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(54) Title: A METHOD OF USING PROTEASOME INHIBITORS IN COMBINATION WITH HISTONE DEACETYLASE INHIBITORS TO TREAT CANCER

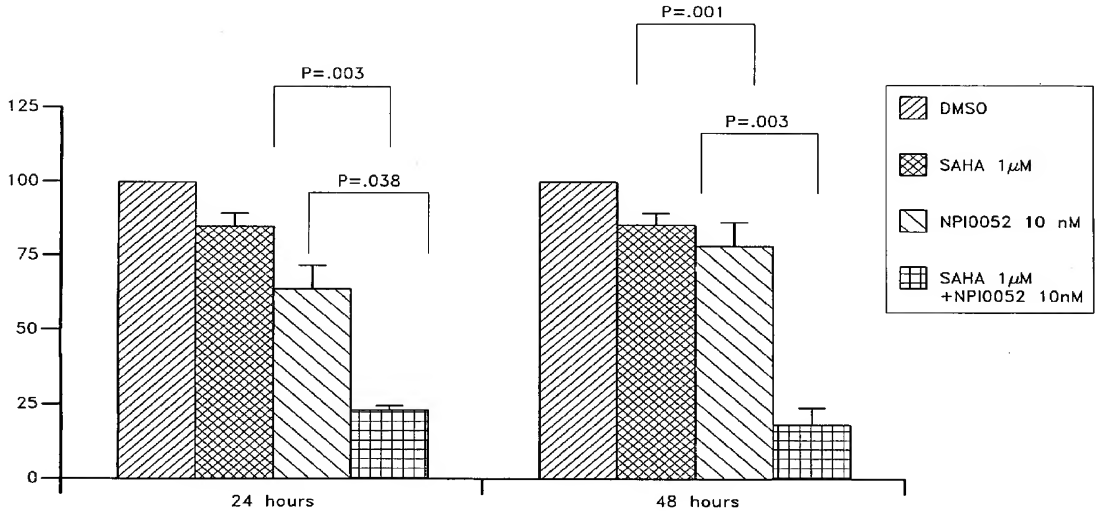

FIG. 9

(57) Abstract: Disclosed are methods of treating cancer comprising administering to the animal, a therapeutically effective amount of proteasome inhibitors and one or more histone deacetylase inhibitor. The animal is a mammal, preferably a human or a rodent.

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A METHOD OF USING PROTEASOME INHIBITORS IN COMBINATION WITH HISTONE DEACETYLASE INHIBITORS TO TREAT CANCER

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to certain compounds and to methods for the preparation and the use of certain compounds in the fields of chemistry and medicine.

Description of the Related Art

[0002] Cancer is a leading cause of death in the United States. Despite significant efforts to find new approaches for treating cancer, the primary treatment options remain surgery, chemotherapy and radiation therapy, either alone or in combination. Surgery and radiation therapy, however, are generally useful only for fairly defined types of cancer, and are of limited use for treating patients with disseminated disease. Chemotherapy is the method that is generally useful in treating patients with metastatic cancer or diffuse cancers such as leukemias. Although chemotherapy can provide a therapeutic benefit, it often fails to result in cure of the disease due to the patient's cancer cells becoming resistant to the chemotherapeutic agent. Due, in part, to the likelihood of cancer cells becoming resistant to a chemotherapeutic agent, such agents are commonly used in combination to treat patients.

[0003] Similarly, infectious diseases caused, for example, by bacteria, fungi and protozoa are becoming increasingly difficult to treat and cure. For example, more and more bacteria, fungi and protozoa are developing resistance to current antibiotics and chemotherapeutic agents. Examples of such microbes include *Bacillus*, *Leishmania*, *Plasmodium* and *Trypanosoma*.

[0004] Furthermore, a growing number of diseases and medical conditions are classified as inflammatory diseases. Such diseases include conditions such as asthma to cardiovascular diseases. These diseases continue to affect larger and larger numbers of people worldwide despite new therapies and medical advances.

[0005] Therefore, a need exists for additional chemotherapeutics, anti-microbial agents, and anti-inflammatory agents to treat cancer, inflammatory diseases and infectious

disease. A continuing effort is being made by individual investigators, academia and companies to identify new, potentially useful chemotherapeutic and anti-microbial agents.

[0006] Marine-derived natural products are a rich source of potential new anti-cancer agents and anti-microbial agents. The oceans are massively complex and house a diverse assemblage of microbes that occur in environments of extreme variations in pressure, salinity, and temperature. Marine microorganisms have therefore developed unique metabolic and physiological capabilities that not only ensure survival in extreme and varied habitats, but also offer the potential to produce metabolites that would not be observed from terrestrial microorganisms (Okami, Y. 1993 *J Mar Biotechnol* **1**:59). Representative structural classes of such metabolites include terpenes, peptides, polyketides, and compounds with mixed biosynthetic origins. Many of these molecules have demonstrable anti-tumor, anti-bacterial, anti-fungal, anti-inflammatory or immunosuppressive activities (Bull, A.T. *et al.* 2000 *Microbiol Mol Biol Rev* **64**:573; Cragg, G.M. & D.J. Newman 2002 *Trends Pharmacol Sci* **23**:404; Kerr, R.G. & S.S. Kerr 1999 *Exp Opin Ther Patents* **9**:1207; Moore, B.S 1999 *Nat Prod Rep* **16**:653; Faulkner, D.J. 2001 *Nat Prod Rep* **18**:1; Mayer, A. M. & V.K. Lehmann 2001 *Anticancer Res* **21**:2489), validating the utility of this source for isolating invaluable therapeutic agents. Further, the isolation of novel anti-cancer and anti-microbial agents that represent alternative mechanistic classes to those currently on the market will help to address resistance concerns, including any mechanism-based resistance that may have been engineered into pathogens for bioterrorism purposes.

Summary of the Invention

[0007] The embodiments disclosed herein generally relate to chemical compounds, including heterocyclic compounds and analogs thereof. Some embodiments are directed to the use of compounds as proteasome inhibitors.

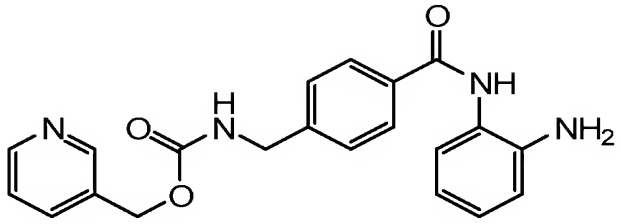
[0008] In other embodiments, the compounds are used to treat neoplastic diseases, for example, to inhibit the growth of tumors, cancers and other neoplastic tissues. The methods of treatment disclosed herein can be employed with any patient suspected of carrying tumorous growths, cancers, or other neoplastic growths, either benign or malignant ("tumor" or "tumors" as used herein encompasses tumors, cancers, disseminated neoplastic

cells and localized neoplastic growths). Examples of such growths include but are not limited to breast cancers; hematologic malignancies including lymphomas, such as Hodgkin's lymphoma and non-hodgkin's lymphoma, leukemias, and multiple myelomas; osteosarcomas, angiosarcomas, fibrosarcomas and other sarcomas; leukemias; sinus tumors; ovarian, uretal, bladder, prostate and other genitourinary cancers; colon, esophageal and stomach cancers and other gastrointestinal cancers; rectal cancers; lung cancers (such as large cell carcinoma, small cell carcinoma, large cell carcinoma, squamous cell carcinoma, adenocarcinoma, and bronchioloalveolar carcinoma); lymphomas; myelomas; teratomas; pancreatic cancers; liver cancers; kidney cancers; endocrine cancers; skin cancers; eye cancers; cervical cancers; anal carcinomas; hepatocellular carcinomas; laryngeal cancers; renal cell carcinomas; testicular cancers; thyroid cancers; osteosarcomas; chondrosarcomsa; Kaposi's sarcoma; rhabdomyosarcomas; melanomas; angiomas; and brain or central nervous system (CNS; glioma) cancers. In general, the tumor or growth to be treated can be any tumor or cancer, primary or secondary. Certain embodiments relate to methods of treating neoplastic diseases in animals. The method can include, for example, administering an effective amount of a compound to a patient in need thereof. Other embodiments relate to the use of compounds in the manufacture of a pharmaceutical or medicament for the treatment of a neoplastic disease.

[0009] The compounds can be administered or used in combination with treatments such as chemotherapy, radiation, and biologic therapies. In some embodiments the compounds can be administered or used with a chemotherapeutic agent. Examples of such chemotherapeutics include Alkaloids, alkylating agents, antibiotics, antimetabolites, enzymes, hormones, platinum compounds, immunotherapeutics (antibodies, T-cells, epitopes), BRMs, and the like. Examples include, Vincristine, Vinblastine, Vindesine, Paclitaxel (Taxol), Docetaxel, topoisomerase inhibibitors epipodophyllotoxins (Etoposide (VP-16), Teniposide (VM-26)), Camptothecin, nitrogen mustards (cyclophosphamide), Nitrosoureas, Carmustine, lomustine, dacarbazine, hydroxymethylmelamine, thiotepa and mitocycin C, Dactinomycin (Actinomycin D), anthracycline antibiotics (Daunorubicin, Daunomycin, Cerubidine), Doxorubicin (Adriamycin), Idarubicin (Idamycin), Anthracenediones (Mitoxantrone), Bleomycin (Blenoxane), Plicamycin (Mithramycin, Antifolates (Methotrexate (Folex, Mexate)), purine antimetabolites (6-mercaptopurine (6-

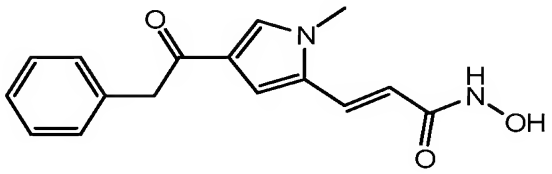
MP, Purinethol) and 6- thioguanine (6-TG). The two major anticancer drugs in this category are 6-mercaptopurine and 6-thioguanine, Chlorodeoxyadenosine and Pentostatin, Pentostatin (2'-deoxycoformycin), pyrimidine antagonists, Avastin, Leucovorin, Oxaliplatin, fluoropyrimidines (5-fluorouracil(Adrucil), 5-fluorodeoxyuridine (FdUrd) (Floxuridine)), Cytosine Arabinoside (Cytosar, ara-C), Fludarabine, Hydroxyurea, glucocorticoids, antiestrogens, tamoxifen, nonsteroidal antiandrogens, flutamide, aromatase inhibitors Anastrozole(Arimidex), Cisplatin, 6-Mercaptopurine and Thioguanine, Methotrexate, Cytoxan, Cytarabine, L-Asparaginase, Steroids: Prednisone and Dexamethasone. Also, proteasome inhibitors such as bortezomib can be used in combination with the instant compounds, for example. Examples of biologics can include agents such as TRAIL antibodies to TRAIL, integrins such as alpha-V-beta-3 ($\alpha V\beta 3$) and / or other cytokine/growth factors that are involved in angiogenesis, VEGF, EGF, FGF and PDGF. In some aspects, the compounds can be conjugated to or delivered with an antibody. The above-described combination methods can be used to treat a variety of conditions, including cancer and neoplastic diseases, inflammation, and microbial infections.

[0010] In some embodiments, the compounds are administered in combination with a histone deacetylase inhibitor (HDACi). In various embodiments, the HDACi is selected from the group consisting of:

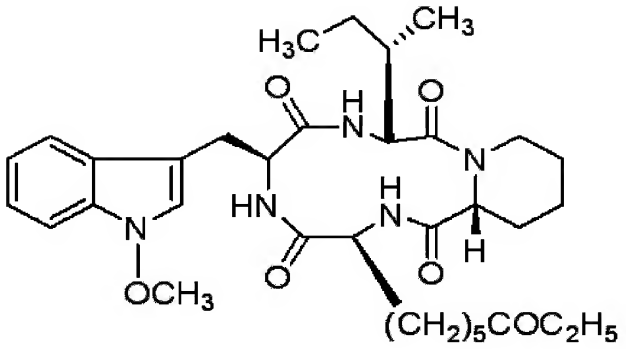


(pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate

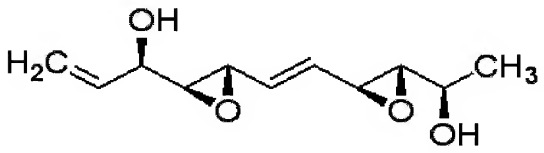
(MS-275 or SNDX-275),



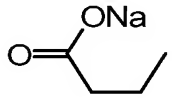
APHA compound 8,



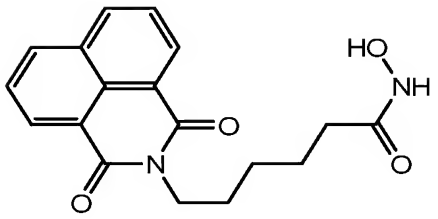
apicidin,



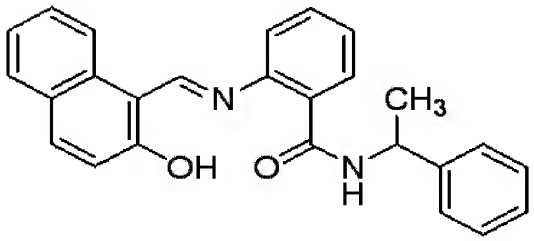
(-)-Depudecin,



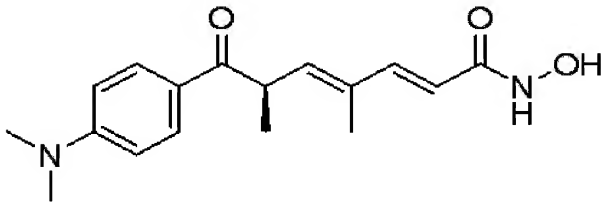
sodium Butyrate,



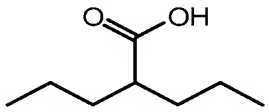
Scriptaid,



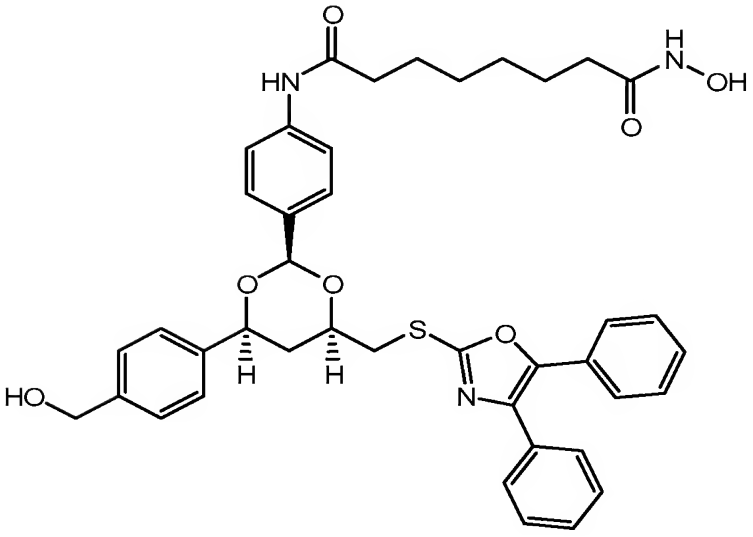
Sirtinol,



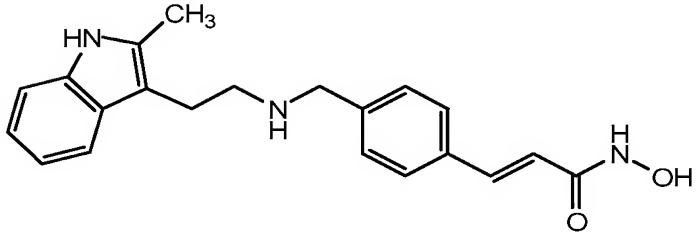
trichostatin A,



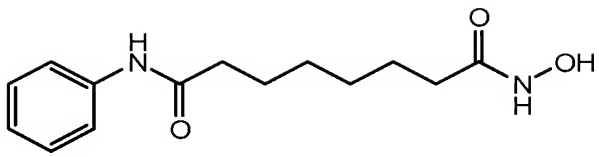
valproic acid,



tubacin,



panobinostat, and

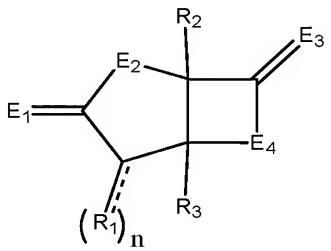


vorinostat (suberoylanilide hydroxamic acid (SAHA)).

[0011] In still other embodiments, the compounds are used to treat inflammatory conditions. Certain embodiments relate to methods of treating inflammatory conditions in animals. The method can include, for example, administering an effective amount of a compound to a patient in need thereof. Other embodiments relate to the use of compounds in the manufacture of a pharmaceutical or medicament for the treatment of inflammation.

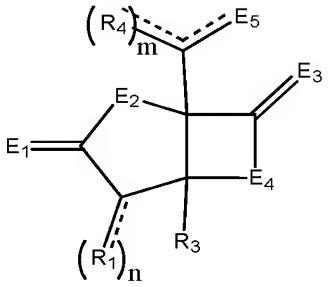
[0012] In certain embodiments, the compounds are used to treat infectious diseases. The infectious agent can be a microbe, for example, bacteria, fungi, protozoans, and microscopic algae, or viruses. Further, the infectious agent can be *B. anthracis* (anthrax). In some embodiments the infectious agent is a parasite. For example, the infectious agent can be *Plasmodium*, *Leishmania*, and *Trypanosoma*. Certain embodiments relate to methods of treating infectious agents in animals. The method can include, for example, administering an effective amount of a compound to a patient in need thereof. Other embodiments relate to the use of compounds in the manufacture of a pharmaceutical or medicament for the treatment of infectious agents.

[0013] The present embodiments provide methods of treating cancer comprising administering to an animal a compound having the structure of any one of Formulas I and II, or a pharmaceutically acceptable salt or pro-drug thereof:



Formula I

and



Formula II

[0014] in combination with a histone deacetylase inhibitor (HDACi);

[0015] wherein:

[0016] the dashed lines represent a single or a double bond;

[0017] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0018] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0019] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0020] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0021] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

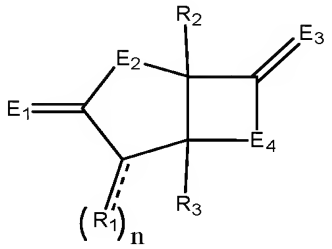
[0022] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

[0023] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

[0024] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl,

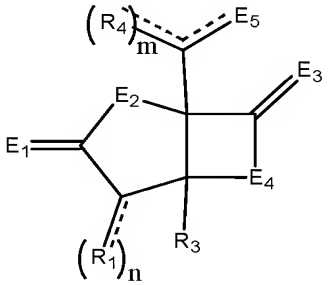
amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

[0025] The present embodiments provide pharmaceutical compositions comprising a histone deacetylase inhibitor (HDACi) and a compound of any one of Formulas I and II:



Formula I

and



Formula II

[0026] wherein:

[0027] the dashed lines represent a single or a double bond;

[0028] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0029] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0030] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0031] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl,

alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0032] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

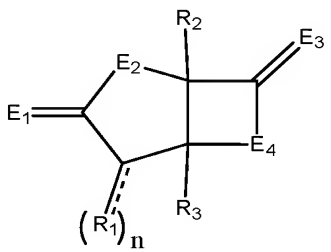
[0033] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

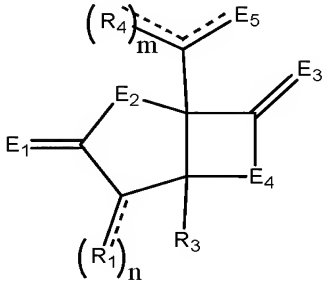
[0034] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

[0035] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

[0036] Other embodiments relate to methods of treating a neoplastic disease in an animal. The methods can include, for example, administering to the animal, a therapeutically effective amount of a compound of a formula selected from Formulae I and II, and pharmaceutically acceptable salts and pro-drug esters thereof.

[0037] The present embodiments provide methods of inhibiting the growth of a cancer cell comprising contacting the cell with a HDACi and a compound having the structure of any one of Formulas I and II:





Formula I and Formula II

[0038] wherein:

[0039] the dashed lines represent a single or a double bond;

[0040] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0041] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0042] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0043] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0044] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

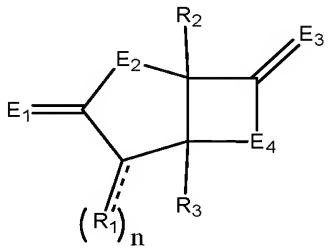
[0045] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

[0046] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

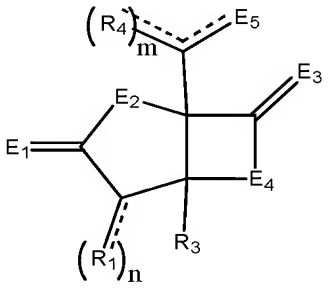
[0047] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl,

cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

[0048] The present embodiments provide methods of inducing apoptosis of a cancer cell comprising contacting the cell with a HDACi and a compound having the structure of any one of Formulas I and II:



Formula I and



Formula II

[0049] wherein:

[0050] the dashed lines represent a single or a double bond;

[0051] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0052] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0053] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0054] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl,

alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0055] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

[0056] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

[0057] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

[0058] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

Brief Description of the Drawings

[0059] The accompanying drawings, which are incorporated in and form part of the specification, merely illustrate certain preferred embodiments of the present invention. Together with the remainder of the specification, they are meant to serve to explain preferred modes of making certain compounds of the invention to those of skilled in the art. In the drawings:

[0060] Fig. 1 shows Salinosporamide A (NPI-0052) inhibits the proteolytic activities of the 20S proteasome in leukemia cell lines.

[0061] Fig. 2 shows *in vitro* and *in vivo* effects of Salinosporamide A (NPI-0052).

[0062] Fig. 3 shows Salinosporamide A (NPI-0052) induced apoptosis through caspase activation and mitochondrial perturbations.

[0063] Fig. 4 shows the role of caspase-8 and FADD in Salinosporamide A (NPI-0052) induced apoptosis.

[0064] Fig. 5 shows the free radical scavenger, NAC, protects from Salinosporamide A (NPI-0052) induced apoptosis.

[0065] Fig. 6 shows low doses of Salinosporamide A (NPI-0052) and HDAC inhibitors induce synergistic apoptosis.

[0066] Fig. 7 shows schematic representation of the mechanism of action of Salinosporamide A (NPI-0052).

[0067] Fig. 8 shows the effect of the combination of MS-275 with bortezomib and the combination of MS-275 with Salinosporamide A to induce synergistic apoptosis.

[0068] Fig. 9 shows that Salinosporamide A (10 nM) enhances the activity of vorinostat (SAHA, 1 μM) in Hodgkin's HD-LM2 Lymphoma cell lines.

[0069] Fig. 10 shows that Salinosporamide A (50 nM) enhances the activity of vorinostat (SAHA, 5 μM) in Hodgkin's L428 cells lines and that Salinosporamide A (50 nM) enhances the activity of vorinostat (SAHA, 5 μM) in KM-H2 cell lines.

[0070] Fig. 11 shows the effect of combinations of Salinosporamide A (14 nm) with MS-275 (0.5 μM, 2 μM and 3 μM) in a human myeloma cell line (RPMI 8226 MM cells), the effect of combinations of Salinosporamide A (18 nM) with MS-275 (1 μM) and the effect of combinations of Salinosporamide A (16 nM) with MS-275 (1.5 μM).

[0071] Fig. 12 shows the effect of combinations of Salinosporamide A (12 nM, 14 nM and 18 nM) with MS-275 (1.5 μM) in a human myeloma cell line (OPM-1 MM cells), and the effect of combinations of Salinosporamide A (18 nm and 20 nm) with MS-275 (2 μM) in a human myeloma cell line (OPM-1 MM cells).

[0072] Fig. 13 shows the effect of combinations of Salinosporamide A (16 nM and 18 nM) with MS-275 (0.5 μM, 1 μM, 1.5 μM and 2 μM) in a human myeloma cell line (DHL-6 MM cells).

[0073] Fig. 14 shows the effect of combinations of Salinosporamide A (12 nM, 14 nM, and 20 nM) with MS-275 (0.5 μM, 1 μM, 1.5 μM and 2 μM) in a human myeloma cell line (Dox-6 MM cells).

[0074] Fig. 15 shows the effect of combinations of Salinosporamide A (14 nM, 16 nM, and 20 nM) with MS-275 (0.5 μM, 1 μM, 1.5 μM and 2 μM) in a human myeloma cell line (Dox-40 MM cells).

[0075] Fig. 16 shows the effect of combinations of Salinosporamide A (12 nM, 14 nM, 18 nM and 20 nM) with MS-275 (0.5 μM, 1 μM, and 2 μM) in a human myeloma cell line (LR-5 MM cells).

[0076] Fig. 17 shows the effect of combinations of Salinosporamide A (3 nM, 5 nM, and 7 nM) with MS-275 (0.25 μM, 0.5 μM, and 1 μM) in a human myeloma cell line (MM.1R cells.

[0077] Fig. 18A shows activity of Salinosporamide A (10 nM) and vorinostat (5 μM) alone and in combination on SB-2 line.

[0078] Fig. 18B shows activity of Salinosporamide A (10 nM) and vorinostat (5 μM) alone and in combination on WM-266-4 melanoma cell line.

[0079] Fig. 19 shows activity of Salinosporamide A (NPI-0052, 10 nM) and vorinostat (SAHA, 5 μM) alone and in combination on MeWo melanoma cell lines.

[0080] Fig. 20 shows growth inhibition effects of Salinosporamide A (NPI-0052) at doses of 5 nM to 500 nM as single agent or in combination with Vorinostat (SAHA, 2 μM) in various lung cancer cell lines.

[0081] Fig. 21 shows growth inhibition effects of Salinosporamide A (NPI-0052) at doses of 5 nM to 500 nM as single agent or in combination with Vorinostat (2 μM) in various lung cancer cell lines.

[0082] Fig. 22A-H shows isobologram analyses for eight lung carcinoma cell lines treated with Salinosporamide A (NPI-0052) and vorinostat (SAHA).

[0083] Fig. 23 shows that human pancreatic carcinoma cell lines are resistant to treatment with gemcitabine alone, when gemcitabine (1 μM or 10 μM) is used in combination with Salinosporamide A a dosage effect is observed.

[0084] Fig. 24 shows the effect of Salinosporamide A (NPI-0052) with vorinostat (SAHA) increases apoptosis compared to treatment with the individual agents alone.

[0085] Fig. 25 shows HDAC inhibitor MS-275 can decrease mRNA expression of 20S proteasome β subunits in Jurkat cells.

[0086] Fig. 26 shows vorinostat can decrease mRNA expression of 20S proteasomal β5 subunit in Jurkat cells as measured at 12 hours and 18 hours. The expression of β5 mRNA was analyzed by real time PCR.

[0087] Fig. 27 shows MS-275 in combination with Salinosporamide A or bortezomib causes Histone-3 to hyperacetylate in Jurkat T-cells.

[0088] Fig. 28 shows combination of Salinosporamide A with vorinostat causes hyperacetylation of Histone-3 in jurkat cells, the addition of N-acetylcysteine (NAC) inhibits the hyperacetylation of Histone-3.

[0089] Fig. 29 shows histone-3 ubiquitination is not affected by Salinosporamide A (NPI-0052)or the combination of Salinosporamide A (NPI-0052) with MS-275.

[0090] Fig. 30 shows effects of vorinostat pretreatment of human Jurkat ALL cells followed by treatment with bortezomib.

[0091] Fig. 31 shows effects of simultaneous treatment of human Jurkat ALL cells with vorinostat and bortezomib.

[0092] Fig. 32 shows effects of vorinostat pretreatment of human Jurkat ALL cells followed by treatment with Salinosporamide A.

[0093] Fig. 33 shows effects of simultaneous treatment of human Jurkat ALL cells with vorinostat and Salinosporamide A.

[0094] Fig. 34 shows effect of MS-275 and Salinosporamide A in combination and alone on superoxide levels in Jurkat T-cells.

[0095] Fig. 35 shows effect of MS-275 and Salinosporamide A in combination and alone on superoxide levels in Caspase-8 deficient cells.

[0096] Fig. 36 shows vorinostat in combination with Salinosporamide A or bortezomib causes formation of reactive oxygen species (ROS), when N-acetylcysteine (NAC) is included in the combinations the amount of ROS decrease as measured by mean fluorescence.

[0097] Fig. 37 shows N-acetylcysteine (NAC) decreases formation of ROS when combined with vorinostat (Zolinza) and Salinosporamide A but not when combined with vorinostat and bortezomib (Velcade).

[0098] Fig. 38 shows combination of vorinostat (SAHA) and Salinosporamide A (NPI) does not induce apoptosis in cells that are caspase-8 deficient as strongly as cells that are not caspase-8 deficient.

[0099] Fig. 39 shows regulation of NF-κB Activity *in vitro* by vorinostat and Salinosporamide A alone and in combination.

[0100] Fig. 40 shows regulation of NF-κB Activity *in vitro* in human pancreatic carcinoma cells by vorinostat and Salinosporamide A alone and in combination.

[0101] Fig. 41 shows combination of Salinosporamide A and vorinostat exhibit enhanced activity in an orthotopic pancreatic tumor model.

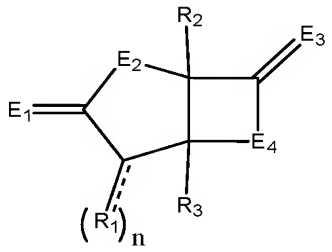
Detailed Description of the Preferred Embodiment

[0102] Numerous references are cited herein. The references cited herein, including the U.S. patents cited herein, are each to be considered incorporated by reference in their entirety into this specification.

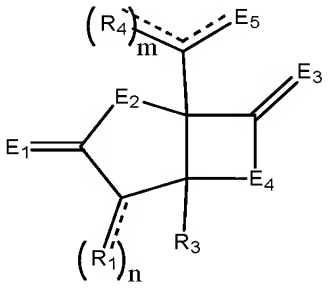
[0103] Mono-therapy with single chemotherapeutic agents and targeted compounds is rarely able to surmount the divergent multi-pathway survival and growth signaling pathways that are critical to the survival of cancer cells. As a result, researchers and clinicians have resorted to investigating the potential of combination therapies. To accurately predict the best potential combinations it is therefore necessary to identify the specific aberrant signaling pathways which are responsible for a cancer phenotype.

[0104] By "co-administration" or use "in combination," it is meant that the two or more agents may be found in the patient's bloodstream at the same time, regardless of when or how they are actually administered. In one embodiment, the agents are administered simultaneously. In one such embodiment, administration in combination is accomplished by combining the agents in a single dosage form. In another embodiment, the agents are administered sequentially. In one embodiment the agents are administered through the same route, such as orally. In another embodiment, the agents are administered through different routes, such as one being administered orally and another being administered i.v. In one advantageous embodiment, the pharmacokinetics of the two or more agents are substantially the same.

[0105] Some embodiments provide a method of treating cancer comprising administering to an animal a compound having the structure of any one of Formulas I and II, or a pharmaceutically acceptable salt or pro-drug ester thereof:



Formula I and



Formula II

[0106] in combination with a histone deacetylase inhibitor (HDACi);

[0107] wherein:

[0108] the dashed lines represent a single or a double bond;

[0109] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0110] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0111] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0112] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0113] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

[0114] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

[0115] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

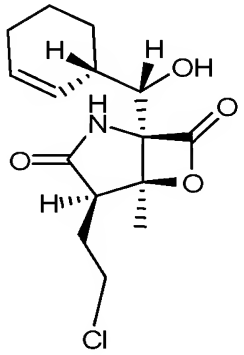
[0116] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

[0117] In some embodiments $\mathbf{E_5}$ can be, for example, OH, O, $\mathbf{OR_{10}}$, S, $\mathbf{SR_{11}}$, $\mathbf{SO_2R_{11}}$, NH, NH_2, NOH, NHOH, $\mathbf{NR_{12}}$, and $\mathbf{NHOR_{13}}$, wherein $\mathbf{R_{10\text{-}13}}$ may separately include, for example, hydrogen, a substituted or unsubstituted of any of the following: alkyl, an aryl, a heteroaryl, and the like. R_3 can be methyl. Furthermore, $\mathbf{R_4}$ may include a cyclohexyl. Also, each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ can be O and $\mathbf{E_2}$ can be NH. Preferably, $\mathbf{R_1}$ can be CH_2CH_2X, wherein **X** is selected from the group consisting of hydrogen, fluorine, chlorine, bromine, and iodine; wherein $\mathbf{R_4}$ may include a cyclohexyl; wherein $\mathbf{R_3}$ can be methyl; and wherein each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ separately can be O and $\mathbf{E_2}$ can be NH. In some embodiments, R_1 can be alkyl optionally substituted with a boranic ester or boranic ester. For example, the boronic ester can be $B(OMethyl)_2$, $B(OEthyl)_2$, $B(OPropyl)_2$, $B(OPhenyl)_2$, and the like.

[0118] In certain embodiments, the cancer can be selected from the group consisting of breast cancer, sarcoma, leukemia, uretal cancer, bladder cancer, colon cancer, rectal cancer, stomach cancer, lung cancer, lymphoma, liver cancer, kidney cancer, endocrine cancer, skin cancer, melanoma, angioma, brain cancer and central nervous system (CNS) cancer. In a typical embodiment, the cancer can be leukemia, lymphoma, and the like. In

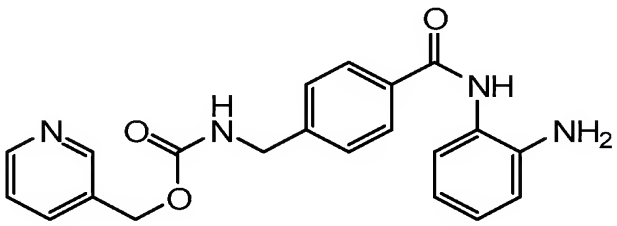
certain embodiments the cancer can comprise a tumor. In a typical embodiment, the tumor can be a refractory solid tumor. In certain embodiments the method can further comprise co-administering a chemotherapeutic agent.

[0119] In certain embodiments, the compound is Salinosporamide A;

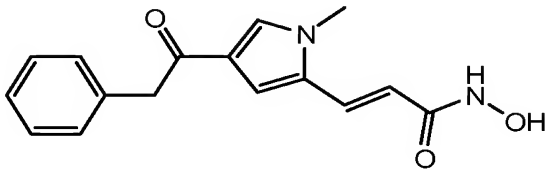


Salinosporamide A

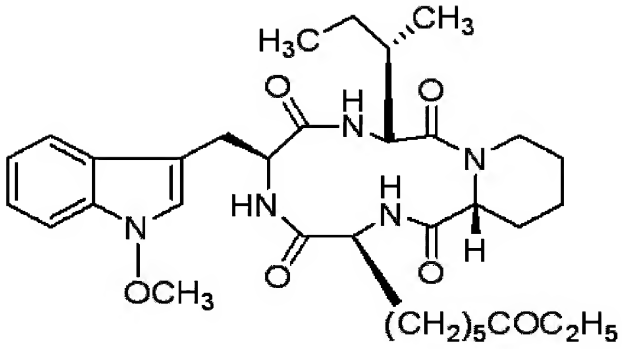
[0120] In certain embodiments, the HDACi and the compound having the structure of any one of Formulas I and II can work in a synergistic manner to treat cancer. In certain embodiments, the HDACi can be selected from the group consisting of:



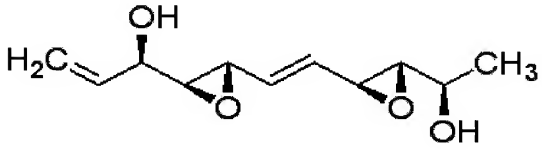
(pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate,



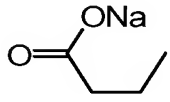
APHA compound 8,



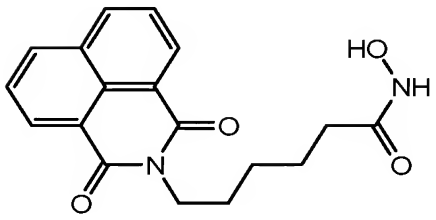
apicidin,



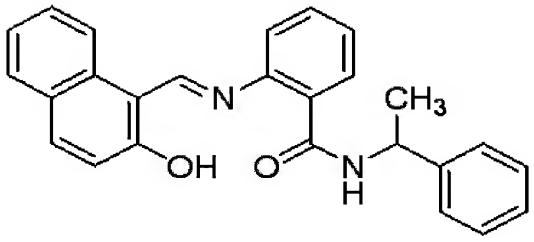
(-)-Depudecin,



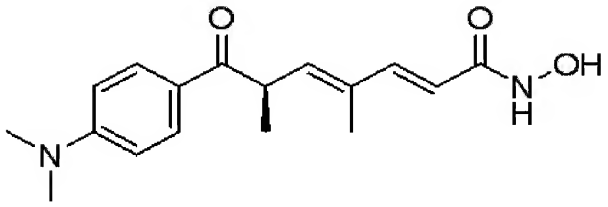
sodium Butyrate,



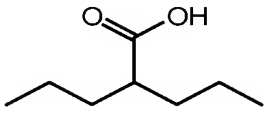
Scriptaid,



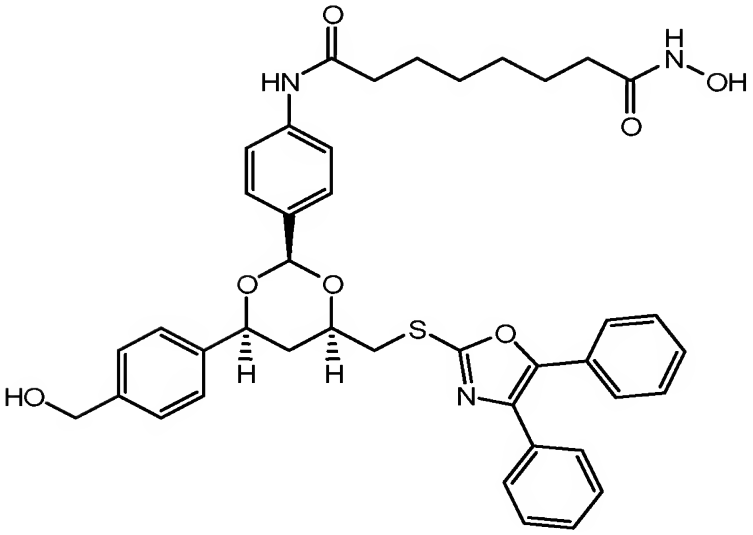
Sirtinol,



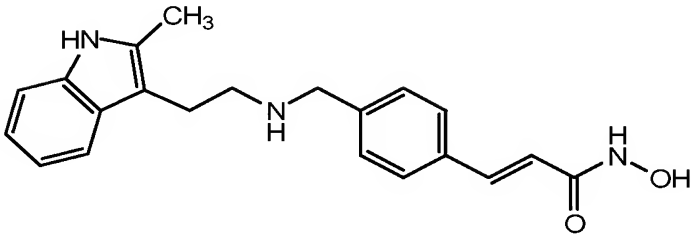
trichostatin A,



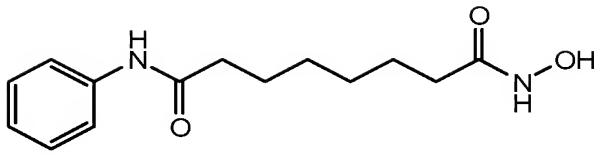
valproic acid,



tubacin,



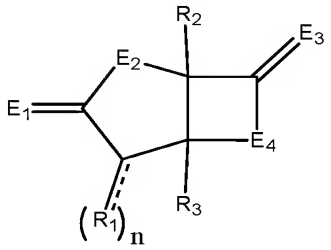
panobinostat, and



vorinostat (suberoylanilide hydroxamic acid (SAHA)).

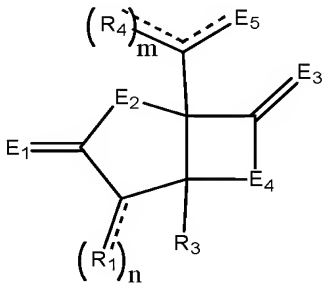
[0121] For example, the HDACi can be (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), valproic acid, vorinostat, and the like.

[0122] Some embodiments provide a pharmaceutical composition comprising a histone deacetylase inhibitor (HDACi) and a compound of any one of Formulas I and II:



Formula I

and



Formula II

[0123] wherein:

[0124] the dashed lines represent a single or a double bond;

[0125] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0126] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0127] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0128] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl,

alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0129] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

[0130] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

[0131] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

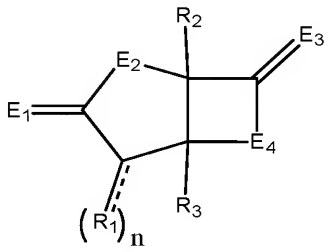
[0132] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

[0133] In some embodiments $\mathbf{E_5}$ can be, for example, OH, O, $\mathbf{OR_{10}}$, S, $\mathbf{SR_{11}}$, $\mathbf{SO_2R_{11}}$, NH, NH_2, NOH, NHOH, $\mathbf{NR_{12}}$, and $\mathbf{NHOR_{13}}$, wherein $\mathbf{R_{10\text{-}13}}$ may separately include, for example, hydrogen, a substituted or unsubstituted of any of the following: alkyl, an aryl, a heteroaryl, and the like. R_3 can be methyl. Furthermore, $\mathbf{R_4}$ may include a cyclohexyl. Also, each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ can be O and $\mathbf{E_2}$ can be NH. Preferably, $\mathbf{R_1}$ can be CH_2CH_2X, wherein **X** is selected from the group consisting of hydrogen, fluorine, chlorine, bromine, and iodine; wherein $\mathbf{R_4}$ may include a cyclohexyl; wherein $\mathbf{R_3}$ can be methyl; and wherein each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ separately can be O and $\mathbf{E_2}$ can be NH. In some embodiments, R_1 can be alkyl optionally substituted with a boranic ester or boranic ester. For example, the boronic ester can be $B(OMethyl)_2$, $B(OEthyl)_2$, $B(OPropyl)_2$, $B(OPhenyl)_2$, and the like.

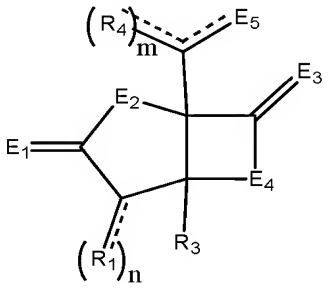
[0134] In a typical embodiment the compound is Salinosporamide A. In certain embodiments the HDACi can be selected from the group consisting of (pyridin-3-yl)methyl

4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), APHA compound 8, Apicidin, (−)-Depudecin, sodium butyrate, scriptaid, sirtinol, trichostatin A, valproic acid, vorinostat and the like. For example, the HDACi can be (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), HDACi is valproic acid, vorinostat, and the like.

[0135] Some embodiments provide a method of inhibiting the growth of a cancer cell comprising contacting the cell with a HDACi and a compound having the structure of any one of Formulas I and II:



Formula I and



Formula II

[0136] wherein:

[0137] the dashed lines represent a single or a double bond;

[0138] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0139] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0140] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0141] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl,

alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0142] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

[0143] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

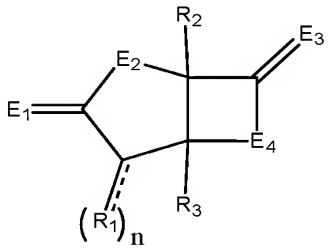
[0144] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

[0145] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

[0146] In some embodiments $\mathbf{E_5}$ can be, for example, OH, O, $\mathbf{OR_{10}}$, S, $\mathbf{SR_{11}}$, $\mathbf{SO_2R_{11}}$, NH, NH_2, NOH, NHOH, $\mathbf{NR_{12}}$, and $\mathbf{NHOR_{13}}$, wherein $\mathbf{R_{10\text{-}13}}$ may separately include, for example, hydrogen, a substituted or unsubstituted of any of the following: alkyl, an aryl, a heteroaryl, and the like. R_3 can be methyl. Furthermore, $\mathbf{R_4}$ may include a cyclohexyl. Also, each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ can be O and $\mathbf{E_2}$ can be NH. Preferably, $\mathbf{R_1}$ can be CH_2CH_2X, wherein **X** is selected from the group consisting of hydrogen, fluorine, chlorine, bromine, and iodine; wherein $\mathbf{R_4}$ may include a cyclohexyl; wherein $\mathbf{R_3}$ can be methyl; and wherein each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ separately can be O and $\mathbf{E_2}$ can be NH. In some embodiments, $\mathbf{R_1}$ can be alkyl optionally substituted with a boranic ester or boranic ester. Typical boranic ester groups include, but are in no way limited to, $B(OMethyl)_2$, $B(OEthyl)_2$, $B(OPropyl)_2$, $B(OPhenyl)_2$, and the like.

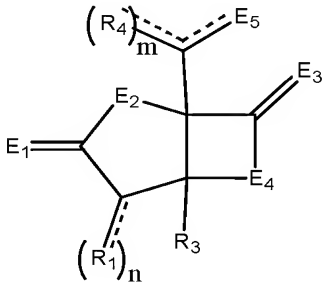
[0147] In a typical embodiment the compound is Salinosporamide A. In certain embodiments the HDACi can be selected from the group consisting of (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), APHA compound 8, Apicidin, (−)-Depudecin, sodium butyrate, scriptaid, sirtinol, trichostatin A, valproic acid, vorinostat and the like. For example, the HDACi can be (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), HDACi is valproic acid, vorinostat, and the like.

[0148] Some embodiments provide a method of inducing apoptosis of a cancer cell comprising contacting the cell with a HDACi and a compound having the structure of any one of Formulas I and II:



Formula I

and



Formula II

[0149] wherein:

[0150] the dashed lines represent a single or a double bond;

[0151] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0152] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0153] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0154] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-

C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0155] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

[0156] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

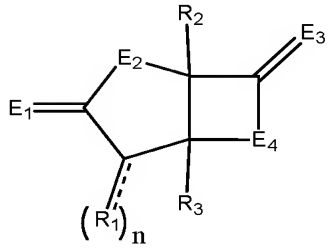
[0157] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

[0158] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

[0159] In some embodiments $\mathbf{E_5}$ can be, for example, OH, O, $\mathbf{OR_{10}}$, S, $\mathbf{SR_{11}}$, $\mathbf{SO_2R_{11}}$, NH, NH_2, NOH, NHOH, $\mathbf{NR_{12}}$, and $\mathbf{NHOR_{13}}$, wherein $\mathbf{R_{10\text{-}13}}$ may separately include, for example, hydrogen, a substituted or unsubstituted of any of the following: alkyl, an aryl, a heteroaryl, and the like. $\mathbf{R_3}$ can be methyl. Furthermore, $\mathbf{R_4}$ may include a cyclohexyl. Also, each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ can be O and $\mathbf{E_2}$ can be NH. Preferably, $\mathbf{R_1}$ can be CH_2CH_2X, wherein **X** is selected from the group consisting of hydrogen, fluorine, chlorine, bromine, and iodine; wherein $\mathbf{R_4}$ may include a cyclohexyl; wherein $\mathbf{R_3}$ can be methyl; and wherein each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ separately can be O and $\mathbf{E_2}$ can be NH. In some embodiments, R_1 can be alkyl optionally substituted with a boranic ester or boranic ester. For example, the boronic ester can be $B(OMethyl)_2$, $B(OEthyl)_2$, $B(OPropyl)_2$, $B(OPhenyl)_2$, and the like.

[0160] In a typical embodiment the compound is Salinosporamide A. In certain embodiments the HDACi can be selected from the group consisting of (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), APHA compound 8, Apicidin, (−)-Depudecin, sodium butyrate, scriptaid, sirtinol, trichostatin A, valproic acid, vorinostat and the like. For example, the HDACi can be (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), HDACi is valproic acid, vorinostat, and the like.

[0161] Some embodiments provide a compound having the structure of Formula I:



Formula I

[0162] wherein:

[0163] the dashed lines represent a single or a double bond;

[0164] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0165] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0166] $\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl,

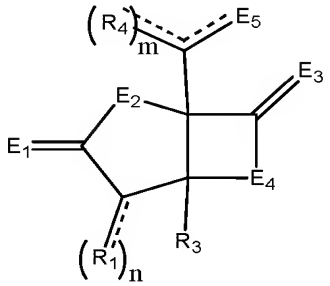
alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0167] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

[0168] each of $\mathbf{E_1}$, $\mathbf{E_3}$, and $\mathbf{E_4}$ is an optionally substituted heteroatom; and

[0169] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group.

[0170] In some embodiments, compounds having the structure of Formula I have the structure of Formula II:



Formula II

[0171] wherein:

[0172] the dashed lines represent a single or a double bond;

[0173] each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

[0174] **n** is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

[0175] **m** is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

[0176] $\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

[0177] each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

[0178] $\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

[0179] each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

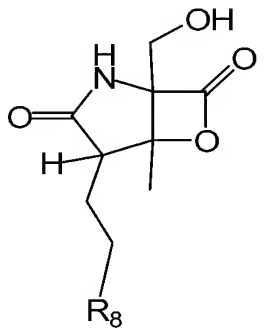
[0180] In some embodiments **n** can be equal to 1, while in others it can be equal to 2. When **n** is equal to 2, the substituents can be the same or can be different. Furthermore, in some embodiments $\mathbf{R_3}$ is not a hydrogen. In some embodiments **m** can be equal to 1 or 2, and when m is equal to 2, $\mathbf{R_4}$ can be the same or different.

[0181] In some embodiments $\mathbf{E_5}$ can be, for example, OH, O, $\mathbf{OR_{10}}$, S, $\mathbf{SR_{11}}$, $\mathbf{SO_2R_{11}}$, NH, NH_2, NOH, NHOH, $\mathbf{NR_{12}}$, and $\mathbf{NHOR_{13}}$, wherein $\mathbf{R_{10\text{-}13}}$ may separately include, for example, hydrogen, a substituted or unsubstituted of any of the following: alkyl, an aryl, a heteroaryl, and the like. $\mathbf{R_3}$ can be methyl. Furthermore, $\mathbf{R_4}$ may include a cyclohexyl. Also, each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ can be O and $\mathbf{E_2}$ can be NH. Preferably, $\mathbf{R_1}$ can be CH_2CH_2X, wherein **X** is selected from the group consisting of hydrogen, fluorine, chlorine, bromine, and iodine; wherein $\mathbf{R_4}$ may include a cyclohexyl; wherein $\mathbf{R_3}$ can be methyl; and wherein each of $\mathbf{E_1}$, $\mathbf{E_3}$ and $\mathbf{E_4}$ separately can be O and $\mathbf{E_2}$ can be NH. In some embodiments, R_1 can be alkyl optionally substituted with a boranic ester or boranic ester. For example, the boronic ester can be $B(OMethyl)_2$, $B(OEthyl)_2$, $B(OPropyl)_2$, $B(OPhenyl)_2$, and the like.

[0182] In some embodiments, $\mathbf{R_2}$ is not cyclohex-2-enyl carbinol when one of the $\mathbf{R_1}$ substituents is ethyl or chloroethyl and $\mathbf{R_3}$ is methyl.

[0183] In some embodiments, $\mathbf{R_1}$ can be an optionally substituted C_1 to C_5 alkyl. For example, $\mathbf{R_1}$ can be methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl and the like. In some embodiments, R_1 is not a substituted or unsubstituted, unbranched C_6 alkyl.

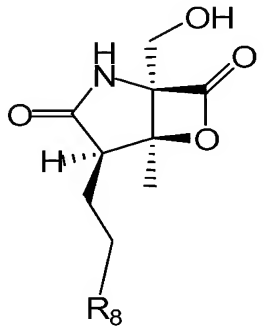
[0184] In a typical embodiment, E_5 can be OH. For example, the compound may have the following Formula I-1:



I-1

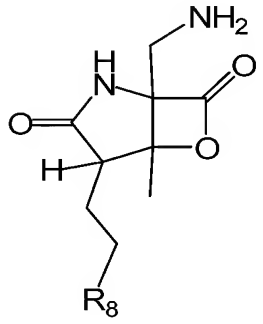
[0185] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0186] As an example, Formula I-1 may have the following stereochemistry:



[0187] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

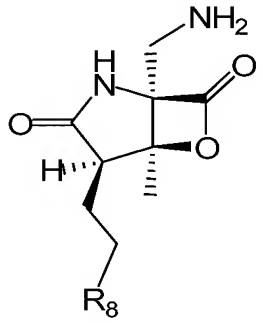
[0188] Still a further exemplary compound of Formula II is a compound having the following Formula I-2:



I-2

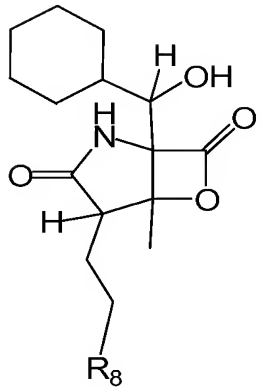
[0189] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0190] For example, Formula I-2 may have the following stereochemistry:



[0191] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

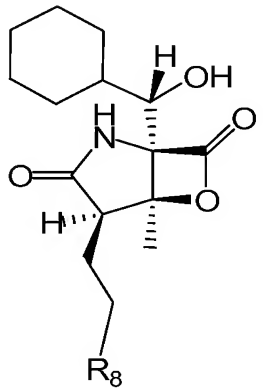
[0192] An exemplary compound of Formula II can heve the following Formula II-1:



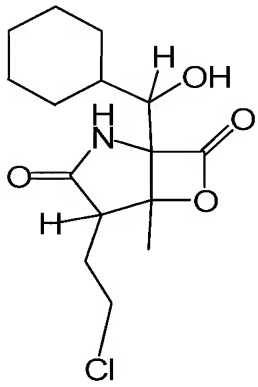
II-1

[0193] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

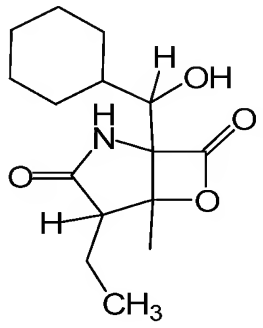
[0194] Exemplary stereochemistry can be as follows:



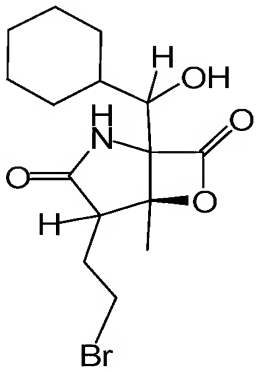
[0195] In some embodiments, the compound of Formula I can have any of the following structures of Foumulae II-2, II-3, and II-4:



II-2

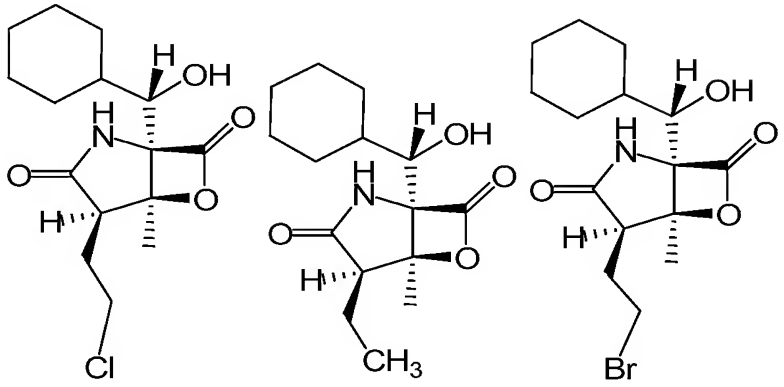


II-3

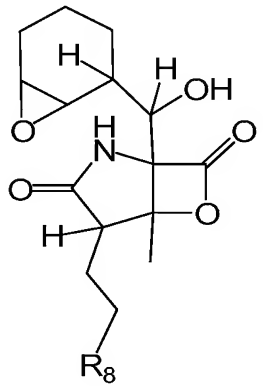


II-4

[0196] The following is exemplary stereochemistry for compounds having the structures of Foumulae II-2, II-3, and II-4, respectively:



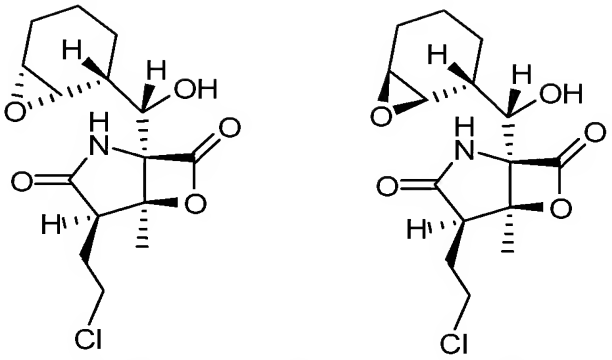
[0197] In other embodiments wherein R_4 may include a 7-oxa-bicyclo[4.1.0]hept-2-yl). An exemplary compound of Formula I is the following Formula II-5:



II-5.

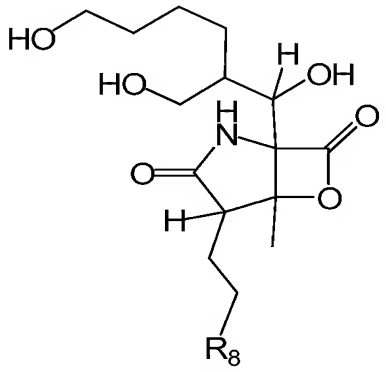
[0198] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0199] The following are examples of compounds of Formula II-5 having the structures of Formulae II-5A and II-5B:



II-5A and **II-5B**.

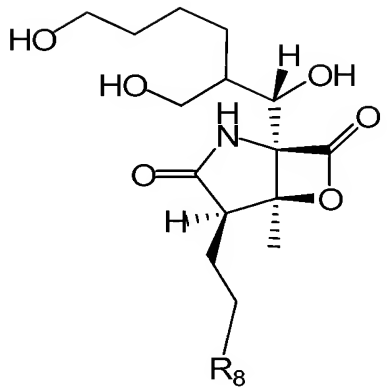
[0200] In still further embodiments, at least one R_4 may include an optionally substituted branched alkyl. For example, a compound of Formula I can be the following Formula II-6:



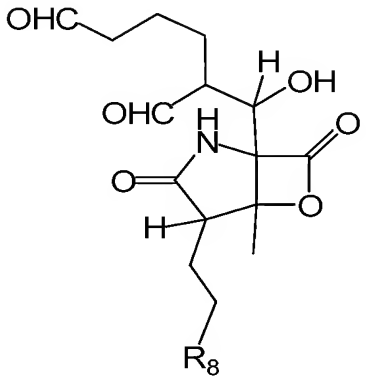
II-6

[0201] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0202] The following is exemplary stereochemistry for a compound of Formula II-6:



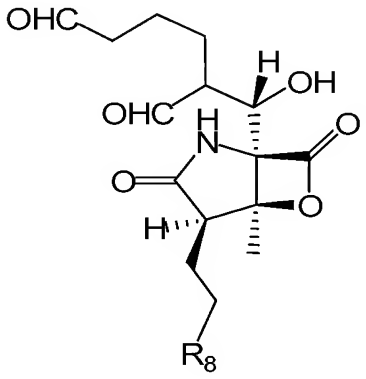
[0203] As another example, the compound of Formula I can be the following Formula II-7:



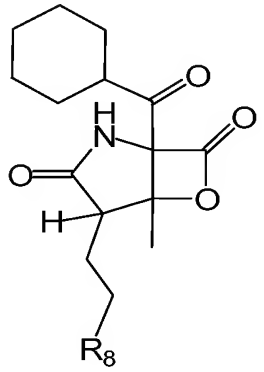
II-7

[0204] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0205] The following is exemplary stereochemistry for a compound having the structure of Formula II-7:



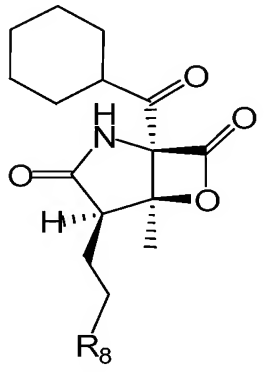
[0206] In other embodiments, at least one R_4 can be an optionally substituted cycloalkyl and E_5 can be an oxygen. For example, R_4 can be cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, and the like. An exemplary compound of Formula I can have the structure of Formula II-8:



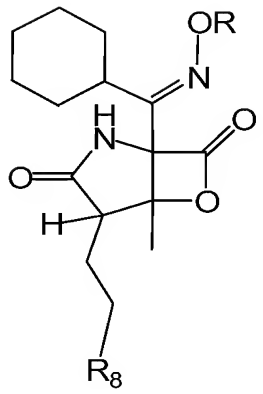
II-8

[0207] In some embodiments, R_8 can be, for example, hydrogen (**II-8A**), fluorine (**II-8B**), chlorine (**II-8C**), bromine (**II-8D**) and iodine (**II-8E**).

[0208] The following is exemplary stereochemistry for a compound having the structure of Formula II-8:



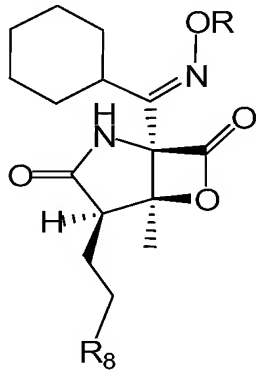
[0209] In some embodiments E5 can be an amine oxide, giving rise to an oxime. An exemplary compound of Formula I has the following structure of Formula II-9:



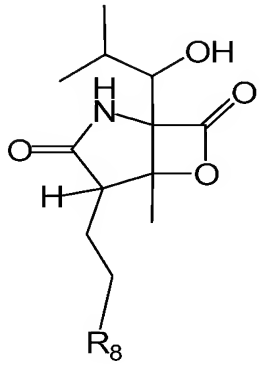
II-9

[0210] R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine; R can be a hydrogen, or an optionally substituted substituent selected from the group consisting of alkyl, aryl, heteroaryl, and the like.

[0211] The following is exemplary stereochemistry for a compound having the structure of Formula II-9:



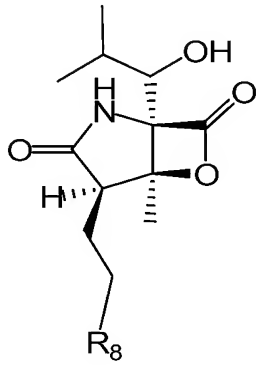
[0212] A further exemplary compound of Formula I has the following structure of Formula II-10:



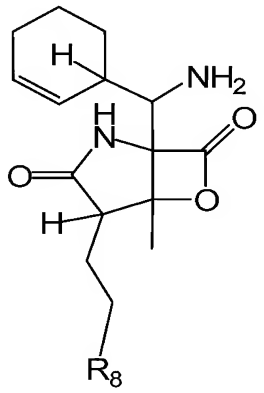
II-10

[0213] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0214] The following is exemplary stereochemistry for a compound having the structure of Formula II-10:



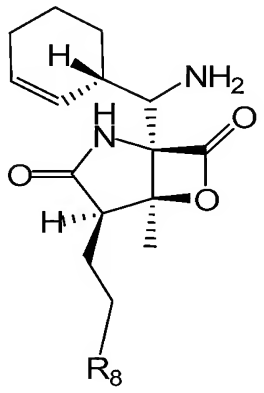
[0215] In some embodiments, E_5 can be NH_2. An exemplary compound of Formula I has the following structure of Formula II-11:



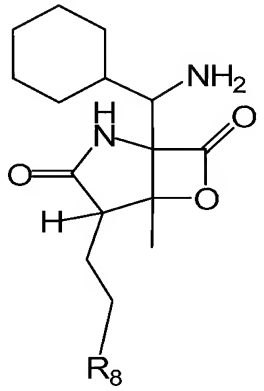
II-11

[0216] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0217] The following is exemplary stereochemistry for a compound having the structure of Formula II-11:



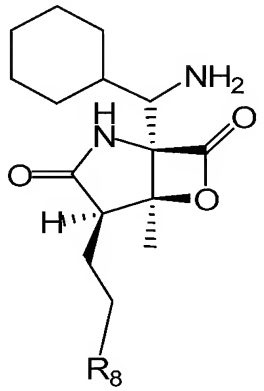
[0218] In some embodiments, at least one R_4 can be an optionally substituted cycloalkyl and E_5 can be NH_2. For example, R_4 can be cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, and the like. An exemplary compound of Formula I has the following structure of Formula II-12:



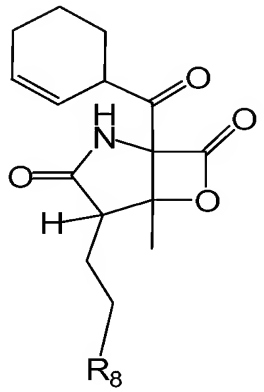
II-12

[0219] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0220] The following is exemplary stereochemistry for a compound having the structure of Formula II-12:



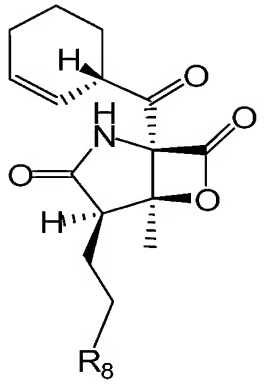
[0221] A further exemplary compound of Formula I has the following structure of Formula II-13:



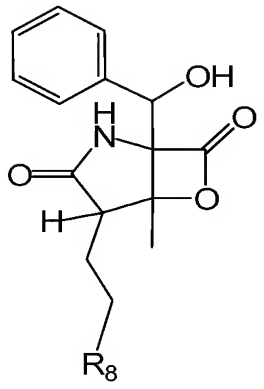
II-13

[0222] R_8 may include, for example, hydrogen (**II-13A**), fluorine (**II-13B**), chlorine (**II-13C**), bromine **(II-13D)** and iodine (**II-13E**).

[0223] The following is exemplary stereochemistry for a compound having the structure of Formula II-13:



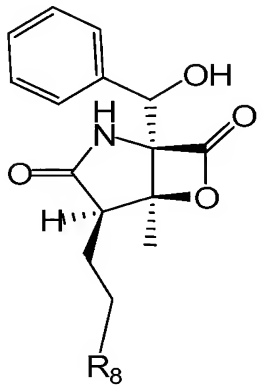
[0224] In another embodiment a compound of Formula I can have the following structure of Formula II-14:



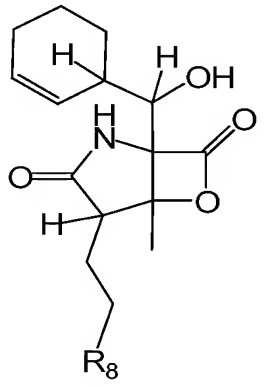
II-14

[0225] For example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine.

[0226] The following is exemplary stereochemistry for a compound having the structure of Formula II-14:



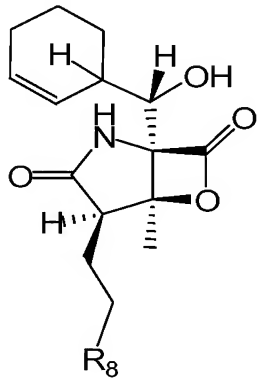
[0227] In another embodiment, for example, the radical $\mathbf{R_4}$ of a compound of Formula II can be an optionally substituted cycloalkene. Furthermore, in some embodiments, the compounds of Formula II may include a hydroxy at $\mathbf{E_5}$, for example. A further exemplary compound of Formula II has the following structure of Formula II-15:



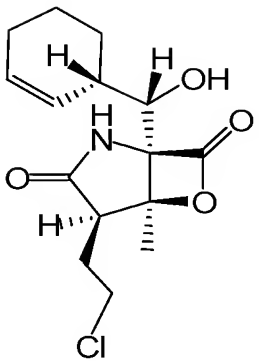
II-15

[0228] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine R_8 may include, for example, hydrogen, fluorine, chlorine, bromine and iodine.

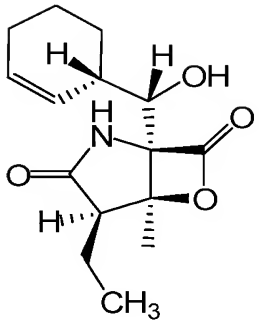
[0229] Exemplary stereochemistry can be as follows:



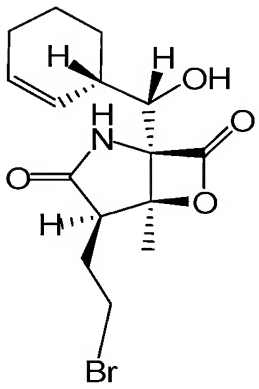
[0230] The following is exemplary stereochemistry for compounds having the structure of Formulae II-16, II-17, II-18, and II-19, respectively:



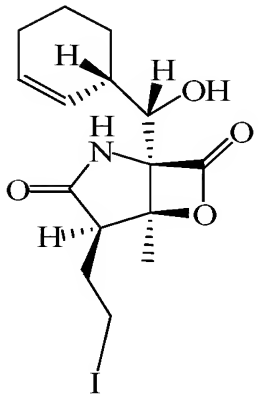
II-16



II-17



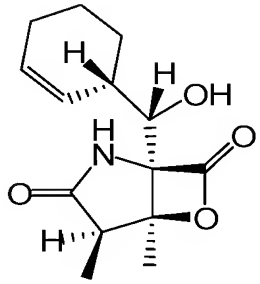
II-18



II-19

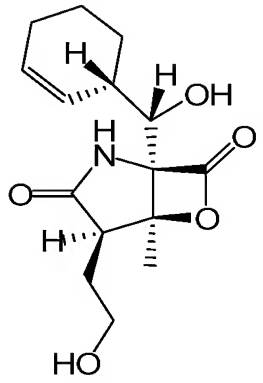
[0231] The compounds of Formulae II-16, II-17, II-18 and II-19 can be obtained by fermentation, synthesis, or semi-synthesis and isolated/purified as set forth below. Furthermore, the compounds of Formulae II-16, II-17, II-18 and II-19 can be used, and are referred to, as "starting materials" to make other compounds described herein.

[0232] In some embodiments, the compounds of Formula I, may include a methyl group as R_1, for example. A further exemplary compound, structure II-20, has the following structure and stereochemistry:



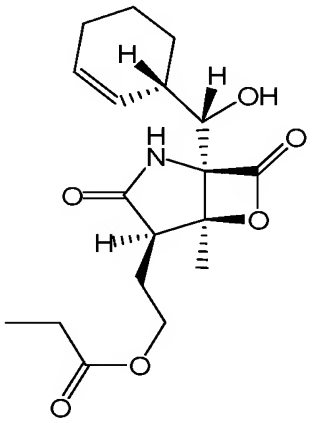
II-20

[0233] In some embodiments, the compounds of Formula I, may include hydroxyethyl as $\mathbf{R_1}$, for example. A further exemplary compound, Formula II-21, has the following structure and stereochemistry:



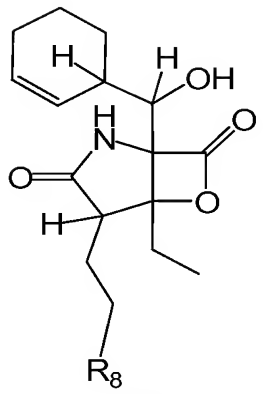
II-21

[0234] In some embodiments, the hydroxyl group of Formula II-21 can be esterified such that $\mathbf{R_1}$ may include ethylpropionate, for example. An exemplary compound, structure II-22, has the following structure and stereochemistry:



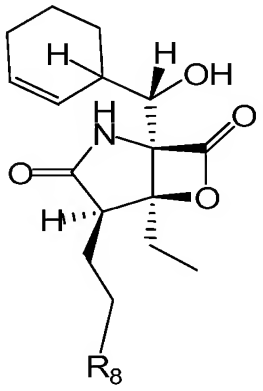
II-22

[0235] In some embodiments, the compounds of Formula I may include an ethyl group as $\mathbf{R_3}$, for example. A further exemplary compound of Formula I has the following structure of Formula II-23:

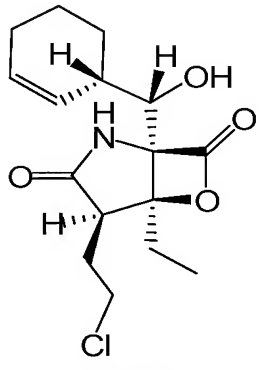


II-23

[0236] For example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine. Exemplary stereochemistry can be as follows:

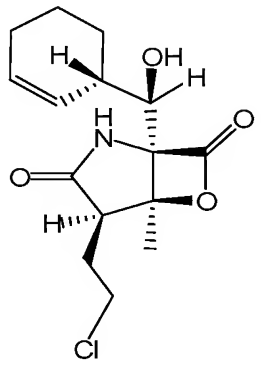


[0237] In some embodiments, the compounds of Formula II-23 may have the following structure and stereochemistry, exemplified by structure of Formula II-24C, where R_8 is chlorine:



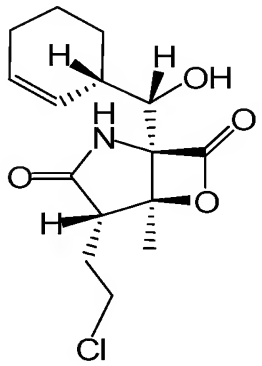
II-24C

[0238] In some embodiments, the compounds of Formula II-15 may have the following stereochemistry, exemplified by the compound of Formula II-25, where R_8 is chlorine:



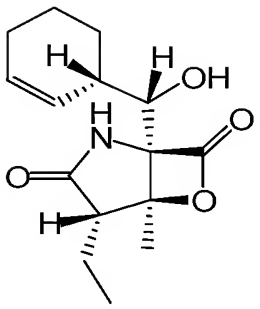
II-25

[0239] In some embodiments, the compound of Formula II-15 may have the following stereochemistry, exemplified by the compound of Formula II-26, where R_8 is chlorine:



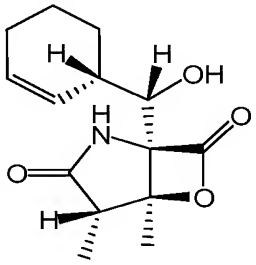
II-26

[0240] In some embodiments, the compound of Formula I may have the following structure and stereochemistry, exemplified by the structure of Formula II-27, where $\mathbf{R_1}$ is ethyl:



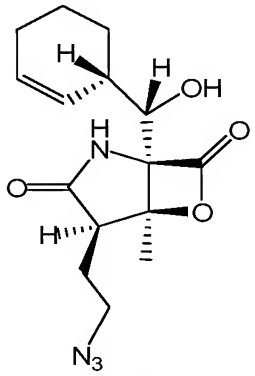
II-27

[0241] In some embodiments, the compound of Formula I may have the following structure and stereochemistry, exemplified by the structure of Formula II-28, where R_1 is methyl:



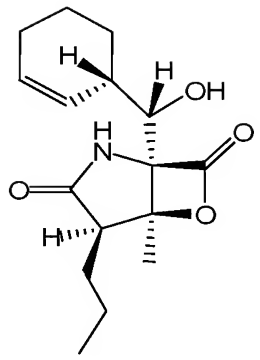
II-28

[0242] In some embodiments, the compounds of Formula I may include azidoethyl as R_1, for example. A further exemplary compound, Formula II-29, has the following structure and stereochemistry:



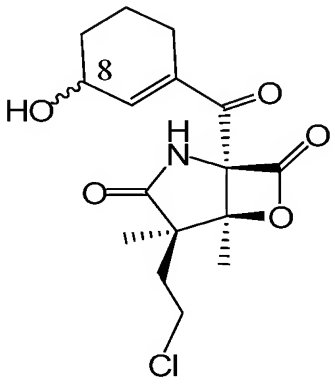
II-29

[0243] In some embodiments, the compounds of Formula I may include propyl as R_1, for example. A further exemplary compound, Formula II-30, has the following structure and stereochemistry:



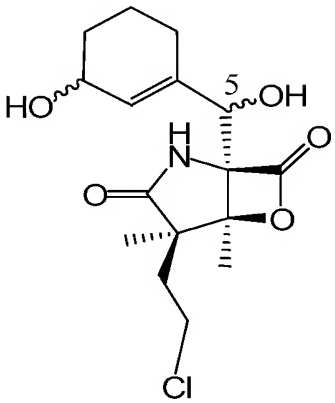
II-30

[0244] Still further exemplary compounds, Formulae II-31 and II-32, have the following structure and stereochemistry:



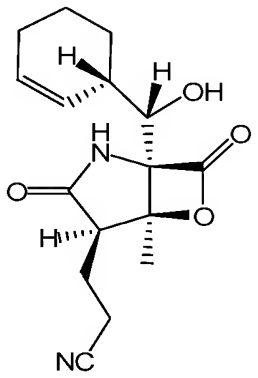
II-31 and **II-32**

[0245] Other exemplary compounds, Formulae II-33, II-34, II-35 and II-36, have the following structure and stereochemistry:



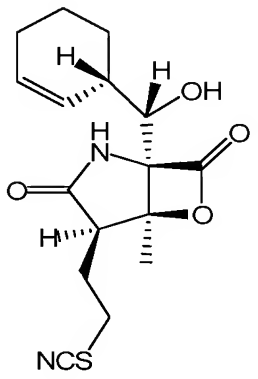
II-33 – II-36

[0246] In some embodiments, the compound of Formula I may include cyanoethyl as $\mathbf{R_1}$; for example, the compound of Formula II-37 has the following structure and stereochemistry:



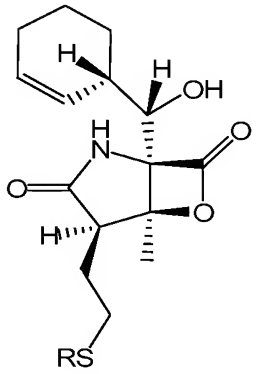
II-37

[0247] In another embodiment, the compound of Formula I may include ethylthiocyanate as $\mathbf{R_1}$; for example, the compound of Formula II-38 has the following structure and stereochemistry:



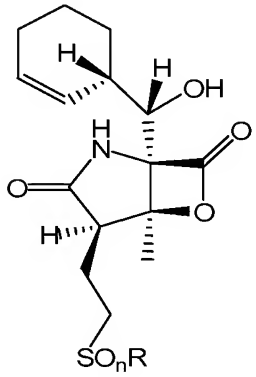
II-38

[0248] In some embodiments, the compounds of Formula I may include a thiol as R_1, for example. A further exemplary compound, Formula II-39, has the following structure and stereochemistry, where R= H, alkyl, aryl, or substituted alkyl or aryl:



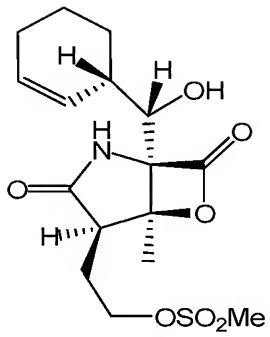
II-39

[0249] In a further exemplary compound, the sulfur of the compound of Formula II-39 can be oxidized to a sulfoxide (n=1) or sulfone (n=2), for example, as in the compound of structure II-40:



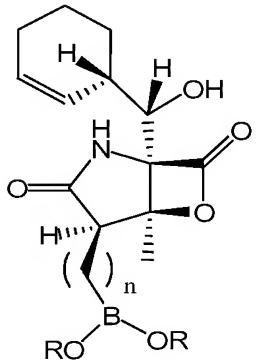
II-40

[0250] In some embodiments, the substituent R_1 of the compound of Formula I may include a leaving group, for example, a halogen, as in compounds of Formulae II-18 or II-19, or another leaving group, such as a sulfonate ester. One example is the methane sulfonate (mesylate) of Formula II-41:



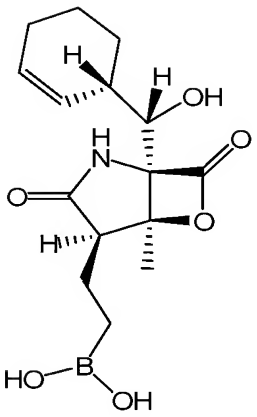
II-41

[0251] In some embodiments, the substituent $\mathbf{R_1}$ of the compound of Formula I may include electron acceptors. The electron acceptor can be, for example, a Lewis acid, such as a boronic acid or ester. An exemplary compound, Formula II-42, has the following structure and stereochemistry, where n = 0, 1, 2, 3, 4, 5, or 6, for example, and where R=H or alkyl, for example:

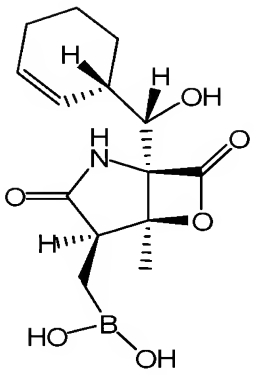


II-42

[0252] Further exemplary compounds of Formula II-42 are the compounds of Formula II-42A, where n=2 and R=H, and the compound of Formula II-42B, where n=1 and R=H:

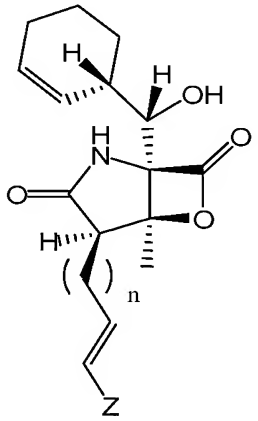


II-42A



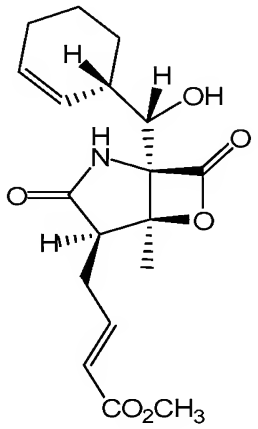
II-42B

[0253] In some embodiments where the substituent R_1 of the compound of Formula I includes an electron acceptor, the electron acceptor can be, for example, a Michael acceptor. An exemplary compound, structure II-43 has the following structure, where n = 0, 1, 2, 3, 4, 5, 6, and where Z is an electron withdrawing group, for example, CHO, COR, COOR, $CONH_2$, CN, NO_2, SOR, SO_2R, etc:



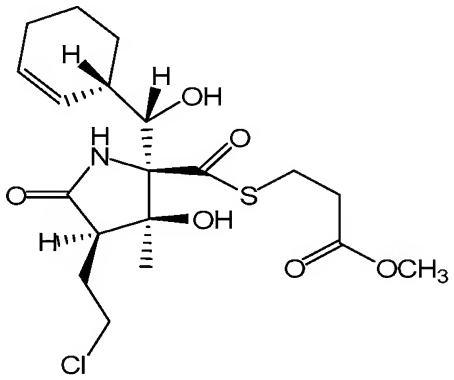
II-43

[0254] A further exemplary compound of Formula II-43 is the compound of structure II-43A, where n=1 and Z=CO_2CH_3:



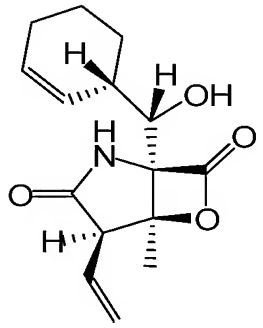
II-43A

[0255] In some embodiments, the compounds can be prodrug esters or thioesters of the compounds of Formula I. For example, the compound of Formula II-44 (a prodrug thioester of the compound of structure II-16) has the following structure and stereochemistry:



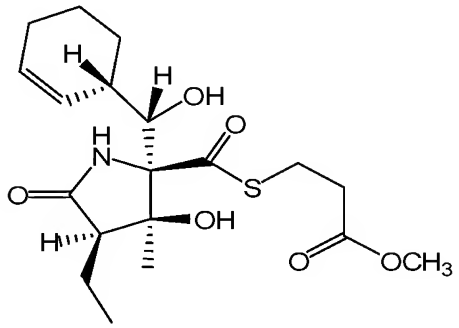
II-44

[0256] In some embodiments, the compounds of Formula I may include an alkenyl group as $\mathbf{R_1}$, for example, ethylenyl. A further exemplary compound, Formula II-46, has the following structure and stereochemistry:



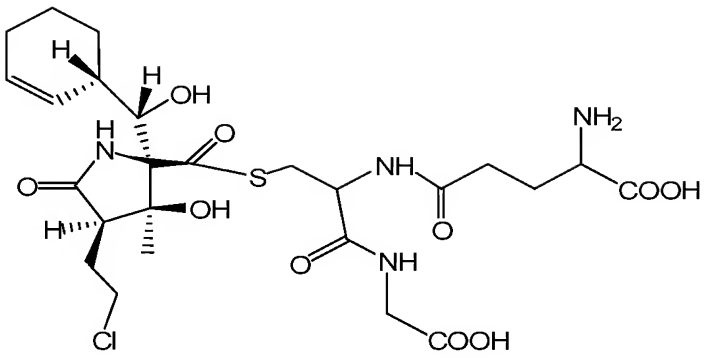
II-46

[0257] In some embodiments, the compounds can be prodrug esters or thioesters of the compounds of Formula I. For example, the compound of Formula II-47 (a prodrug thioester of the compound of structure II-17) has the following structure and stereochemistry:



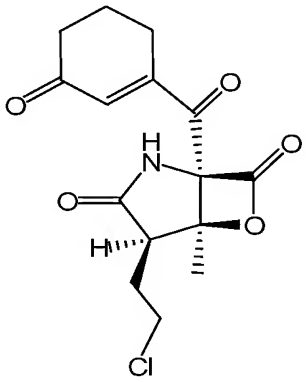
II-47

[0258] In some embodiments, the compounds can be prodrug esters or thioesters of the compounds of Formula I. For example, the compound of Formula II-48 has the following structure and stereochemistry:



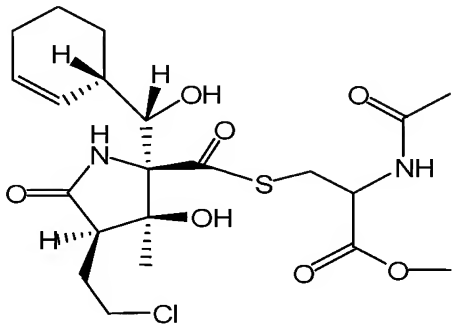
II-48

[0259] Another exemplary compound, structure II-49 has the following structure and stereochemistry:



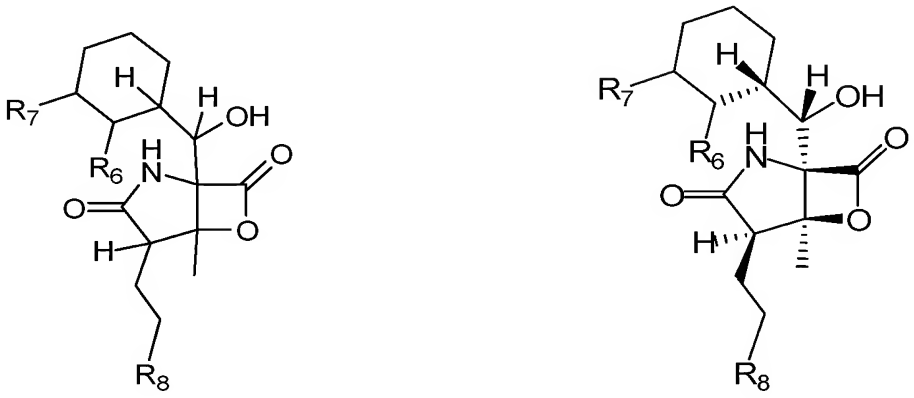
II-49

[0260] In some embodiments, the compound can be prodrug ester or thioester of the compounds of Formula I. For example, the compound of Formula II-50 (prodrug ester of the compound of Formula II-16) has the following structure and stereochemistry:



II-50

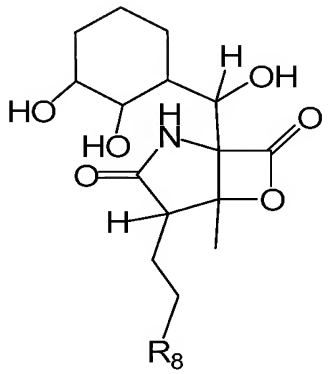
[0261] An exemplary compound of Formula I is the following Formula III-1, with and without exemplary stereochemistry:



III-1

[0262] In some embodiments, for example, R_8 can be selected from the group consisting of hydrogen, fluorine, chlorine, bromine and iodine. The substituent(s) R_6 and R_7 may each separately be selected from a hydrogen, a halogen, a nitro, a cyano, or an optionally substituted substituent selected from the group consisting of C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxy carbonyl, alkoxy carbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, azido, phenyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, and halogenated alkyl including polyhalogenated alkyl. Further, R_6 and R_7 both can be the same or different.

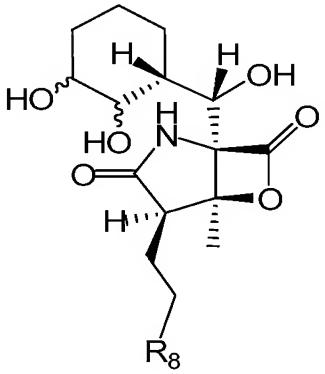
[0263] For example, an exemplary compound of Formula I has the following Formula III-2:



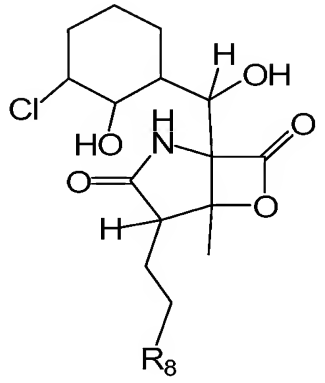
III-2

[0264] R_8 may include, for example, hydrogen, fluorine, chlorine, bromine and iodine.

[0265] Exemplary stereochemistry can be as follows:



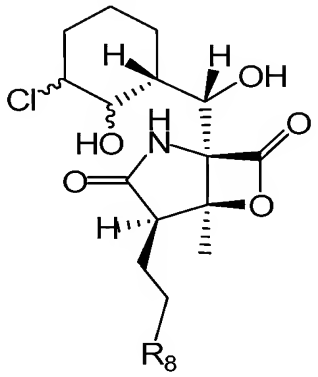
[0266] For example, an exemplary compound of Formula I has the following Formula III-3:



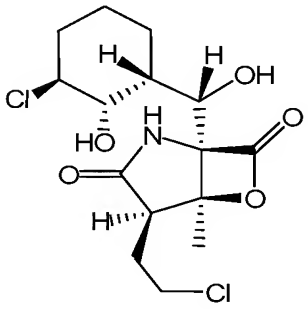
III-3

[0267] R_8 may include, for example, hydrogen (**III-3A**), fluorine (**III-3B**), chlorine (**III-3C**), bromine (**III-3D**) and iodine (**III-3E**).

[0268] Exemplary structure and stereochemistry can be as follows:

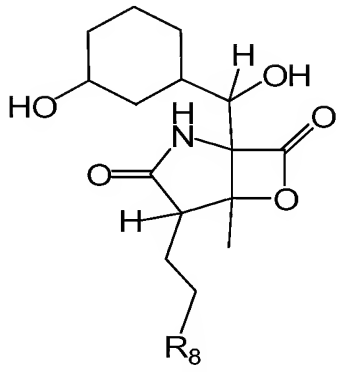


[0269] Additional exemplary structure and stereochemistry can be as follows:



III-3C

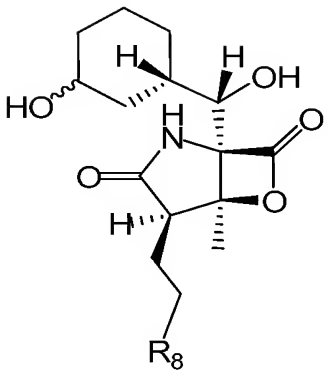
[0270] For example, an exemplary compound of Formula I has the following Formula III-4:



III-4

[0271] R_8 may include, for example, hydrogen, fluorine, chlorine, bromine and iodine.

[0272] Exemplary stereochemistry can be as follows:



[0273] Certain embodiments also provide pharmaceutically acceptable salts and pro-drug esters or thioesters of the compound of Formulae I and II, and provide methods of obtaining and purifying such compounds by the methods disclosed herein.

[0274] The term "pro-drug," especially when referring to a pro-drug ester of the compound of Formula I synthesized by the methods disclosed herein, refers to a chemical derivative of the compound that is rapidly transformed *in vivo* to yield the compound, for example, by hydrolysis in blood or inside tissues. The term "pro-drug ester" refers to derivatives of the compounds disclosed herein formed by the addition of any of several ester- or thioester-forming groups that are hydrolyzed under physiological conditions. Examples of pro-drug ester groups include pivoyloxymethyl, acetoxymethyl, phthalidyl, indanyl and methoxymethyl, as well as other such groups known in the art, including a (5-*R*-2-oxo-1,3-dioxolen-4-yl)methyl group. Other prodrugs can be prepared by preparing a corresponding thioester of the compound, for example, by reacting with an appropriate thiol, such as thiophenol, Cysteine or derivatives thereof, or propanethiol, for example. Other examples of pro-drug ester groups can be found in, for example, T. Higuchi and V. Stella, in "Pro-drugs as Novel Delivery Systems", Vol. 14, A.C.S. Symposium Series, American Chemical Society (1975); and "Bioreversible Carriers in Drug Design: Theory and Application", edited by E. B. Roche, Pergamon Press: New York, 14-21 (1987) (providing examples of esters useful as prodrugs for compounds containing carboxyl groups). Each of the above-mentioned references is hereby incorporated by reference in its entirety.

[0275] The term "pharmaceutically acceptable salt," as used herein, and particularly when referring to a pharmaceutically acceptable salt of a compound, including a compound of Formula I, as produced and synthesized by the methods disclosed herein, refers

to any pharmaceutically acceptable salts of a compound, and preferably refers to an acid addition salt of a compound. Preferred examples of pharmaceutically acceptable salt are the alkali metal salts (sodium or potassium), the alkaline earth metal salts (calcium or magnesium), or ammonium salts derived from ammonia or from pharmaceutically acceptable organic amines, for example C_1-C_7 alkylamine, cyclohexylamine, triethanolamine, ethylenediamine or tris-(hydroxymethyl)-aminomethane. With respect to compounds synthesized by the method of this embodiment that are basic amines, the preferred examples of pharmaceutically acceptable salts are acid addition salts of pharmaceutically acceptable inorganic or organic acids, for example, hydrohalic, sulfuric, phosphoric acid or aliphatic or aromatic carboxylic or sulfonic acid, for example acetic, succinic, lactic, malic, tartaric, citric, ascorbic, nicotinic, methanesulfonic, p-toluensulfonic or naphthalenesulfonic acid.

[0276] Preferred pharmaceutical compositions disclosed herein include pharmaceutically acceptable salts and pro-drugs of the compound of Formula I obtained and purified by the methods disclosed herein. Accordingly, if the manufacture of pharmaceutical formulations involves intimate mixing of the pharmaceutical excipients and the active ingredient in its salt form, then it is preferred to use pharmaceutical excipients which are non-basic, that is, either acidic or neutral excipients.

[0277] It will be also appreciated that the phrase "compounds and compositions comprising the compound," or any like phrase, is meant to encompass compounds in any suitable form for pharmaceutical delivery, as discussed in further detail herein. For example, in certain embodiments, the compounds or compositions comprising the same may include a pharmaceutically acceptable salt of the compound.

[0278] The term "halogen atom," as used herein, means any one of the radio-stable atoms of column 7 of the Periodic Table of the Elements, *i.e.*, fluorine, chlorine, bromine, or iodine.

[0279] The term "alkyl," as used herein, means any unbranched or branched, substituted or unsubstituted, fully saturated (no double or triple bonds) hydrocarbon group. The alkyl group may have 1 to 24 carbon atoms (whenever it appears herein, a numerical range such as "1 to 24" refers to each integer in the given range; *e.g.*, "1 to 24 carbon atoms" means that the alkyl group may consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms,

etc., up to and including 24 carbon atoms, although the present definition also covers the occurrence of the term "alkyl" where no numerical range is designated). The alkyl group may also be a medium size alkyl having 1 to 10 carbon atoms. The alkyl group could also be a lower alkyl having 1 to 5 carbon atoms. The alkyl group of the compounds may be designated as "C_{1-6} alkyl" or similar designations. By way of example only, "C_{1-6} alkyl" indicates that there are one to six carbon atoms in the alkyl chain, i.e., the alkyl chain is selected from the group consisting of methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, t-butyl, pentyl and hexyl. Typical alkyl groups include, but are in no way limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, hexyl, and the like.

[0280] The term "substituted" has its ordinary meaning, as found in numerous contemporary patents from the related art. See, for example, U.S. Patent Nos. 6,509,331; 6,506,787; 6,500,825; 5,922,683; 5,886,210; 5,874,443; and 6,350,759; all of which are incorporated herein in their entireties by reference. Specifically, the definition of substituted is as broad as that provided in U.S. Patent No. 6,509,331, which defines the term "substituted alkyl" such that it refers to an alkyl group, preferably of from 1 to 10 carbon atoms, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyacylamino, cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, thiocyanate, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, azido, boronic acid, boronic ester, --SO-alkyl, --SO-substituted alkyl, --SO-aryl, --SO-heteroaryl, --SO_2-alkyl, --SO_2-substituted alkyl, --SO_2-aryl, --SO_2-heteroaryl, --OSO-alkyl, --OSO-substituted alkyl, --OSO-aryl, --OSO-heteroaryl, --OSO_2-alkyl, --OSO_2-substituted alkyl, --OSO_2-aryl, and --OSO_2-heteroaryl. The other above-listed patents also provide standard definitions for the term "substituted" that are well-understood by those of skill in the art.

[0281] The term "cycloalkyl" as used herein, refers to any non-aromatic hydrocarbon ring, preferably having three to twelve atoms comprising the ring.

[0282] The term "acyl" as used herein, refers to alkyl or aryl groups derived from an oxoacid, with an acetyl group being preferred.

[0283] The term "alkoxycarbonylacyl" as used herein, refers to an acyl group substituted with an alkoxycarbonyl group. Typical alkoxycarbonylacyl groups include, but are in no way limited to, $CH_3OC(O)CH_2C(O)-$, $CH_3CH_2CH_2OC(O)CH_2C(O)-$, 4-ethoxycarbonylbenzoyl−, 4-methoxycarbonylbenzoyl−, 4-propoxycarbonylbenzoyl−, 3-tert-butoxycarbonylbenzoyl−, and the like.

[0284] The term "amino" as used herein, refers to amine radicals, wherein one or both hydrogen atoms are optionally replaced by substituents such as alkyl, and aryl groups. Typical amino groups include, but are in no way limited to, $-NH_2$, −NHMe, −NHEt, $-NHCH_2$phenyl, −N(Me)(phenyl), −N(Et)(Me), −N(Phenyl)(Et), $-N(Et)(CH_2$phenyl), $-N(CH_2$phenyl)(phenyl), and the like.

[0285] The term "aminocarbonyl" and as used herein, refers to a carbonyl substituted with an amino. Typical aminocarbonyl groups include, but are in no way limited to, $-C(O)NH_2$, −C(O)NHMe, −C(O)NHEt, $-C(O)NHCH_2$phenyl, −C(O)N(Me)(phenyl), −C(O)N(Et)(Me), −C(O)N(Phenyl)(Et), $-C(O)N(Et)(CH_2$phenyl), $-C(O)N(CH_2$phenyl)(phenyl), and the like.

[0286] The term "acyloxy" as used herein, refers to an acyl group attached to an oxygen with the oxygen being the attachment point. Typical acyloxy groups include, but are in no way limited to, MeC(O)O−, PhenylC(O)O−, and the like.

[0287] The term "alkenyl" as used herein, means any unbranched or branched, substituted or unsubstituted, unsaturated hydrocarbon including polyunsaturated hydrocarbons, with C_1-C_6 unbranched, mono-unsaturated and di-unsaturated, unsubstituted hydrocarbons being preferred, and mono-unsaturated, di-halogen substituted hydrocarbons being most preferred.

[0288] The term "cycloalkenyl" as used herein, refers to any non-aromatic hydrocarbon ring, preferably having five to twelve atoms comprising the ring and having at least one unsaturated bond.

[0289] The term "heterocycle" or "heterocyclic" refer to any non-aromatic cyclic compound containing one or more heteroatoms. In polycyclic ring systems, the one or more heteroatoms, may be present in only one of the rings. A heterocycle or heterocyclic group

may be substituted or unsubstituted. The substituted heterocycle or heterocyclic group can be substituted with any substituent, including those described above and those known in the art.

[0290] The term "aryl" as used herein, refers to a carbocyclic (all carbon) ring or two or more fused rings (rings that share two adjacent carbon atoms) that have a fully delocalized pi-electron system. Typical aryl groups include, but are in no way limited to, benzene, naphthalene, azulene and the like. An aryl group may be substituted or unsubstituted. The substituted aryls can be substituted with any substituent, including those described above and those known in the art.

[0291] The term "heteroaryl" as used herein, refers to an aromatic heterocyclic group, whether one ring or multiple fused rings. In fused ring systems, the one or more heteroatoms, may be present in only one of the rings. The hetero atom is an element other than carbon, including but not limited to, nitrogen, oxygen and sulfur. Typical heteroaryl groups include, but are in no way limited to, indole, oxazole, benzoxazole, isoxazole, benzisoxazole, thiazole, benzothiazole, isothiazole, imidazole, benzimidazole, pyrazole, pyridazine, pyridine, pyrimidine, purine, pyrazine, pteridine, pyrrole, phenoxazole, oxazole, isoxazole, oxadiazole, benzopyrazole, indazole, quinolizine, cinnoline, phthalazine, quinazoline, quinoxaline, and the like. A heteroaryl group of this invention may be substituted or unsubstituted. The substituted heteroaryls can be substituted with any substituent, including those described above and those known in the art.

[0292] The term "alkoxy" as used herein, refers to any unbranched, or branched, substituted or unsubstituted, saturated or unsaturated ether, with C_1-C_6 unbranched, saturated, unsubstituted ethers being preferred, with methoxy being preferred, and also with dimethyl, diethyl, methyl-isobutyl, and methyl-tert-butyl ethers also being preferred.

[0293] The term "cycloalkoxy" as used herein, refers to any cycloalkyl attached to an oxygen atom with the oxygen being the attachment point to the rest of the molecule.

[0294] The term "arylalkoxy" as used herein, refers to an alkoxy group substituted with an aryl group. For example, arylalkoxy can be methoxy substituted with an aryl group, such as benzyloxy and the like.

[0295] The term "arylalkoxycarbonyl" as used herein, refers to an arylalkoxy group attached to a carbonyl group with the carbonyl being the attachment point to the rest of

the molecule. Typical arylalkoxycarbonyl groups include, but are in no way limited to, benzyloxycarbonyl (i.e., Phenyl$CH_2OC(O)-$) and the like.

[0296] The term "cycloalkyl" as used herein, refers to any non-aromatic hydrocarbon ring.

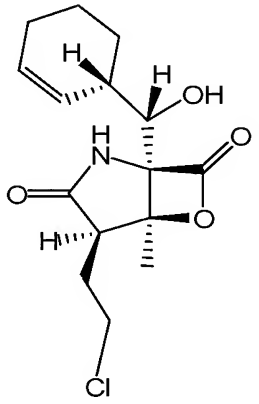
[0297] The term "alkoxycarbonyl" as used herein, refers to any linear, branched, cyclic, saturated, unsaturated, aliphatic or aryl alkoxy attached to a carbonyl group with the carbonyl group being the attachment point to the rest of the molecule. Typical alkoxycarbonyl groups include, but are in no way limited to, ethoxycarbonyl group, propyloxycarbonyl group, isopropyloxycarbonyl group, butoxycarbonyl group, sec-butoxycarbonyl group, tert-butoxycarbonyl group, cyclopentyloxycarbonyl group, cyclohexyloxycarbonyl group, benzyloxycarbonyl group, allyloxycarbonyl group, phenyloxycarbonyl group, pyridyloxycarbonyl group, and the like.

[0298] The term "alkoxycarbonyloxy" as used herein, refers to an alkoxycarbonyl group attached to an oxygen with the oxygen being the attachment point to the rest of the molecule. Typical alkoxycarbonyloxy groups include, but are in no way limited to, $MeOC(O)O-$, methoxycarbonyloxy group, ethoxycarbonyloxy group, propyloxycarbonyloxy group, isopropyloxycarbonyloxy group, butoxycarbonyloxy group, sec-butoxycarbonyloxy group, tert-butoxycarbonyloxy group, cyclopentyloxycarbonyloxy group, cyclohexyloxycarbonyloxy group, allyloxycarbonyloxy group, benzyloxycarbonyloxy group and the like. Additionally, alkoxycarbonyloxy groups refer to aryloxy and heteroaryloxy groups such as, phenyloxycarbonyloxy group, pyridyloxycarbonyloxy group, and the like.

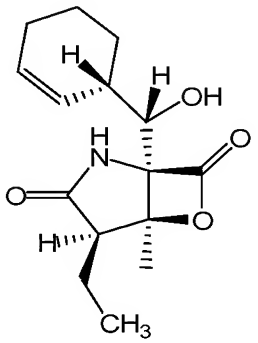
[0299] The terms "pure," "purified," "substantially purified," and "isolated" as used herein refer to the compound of the embodiment being free of other, dissimilar compounds with which the compound, if found in its natural state, would be associated in its natural state. In certain embodiments described as "pure," "purified," "substantially purified," or "isolated" herein, the compound may comprise at least 0.5%, 1%, 5%, 10%, or 20%, and most preferably at least 50% or 75% of the mass, by weight, of a given sample.

[0300] The terms "derivative," "variant," or other similar term refers to a compound that is an analog of the other compound.

[0301] Certain of the compounds of Formula I can be obtained and purified or can be obtained via semi-synthesis from purified compounds as set forth herein. Generally, without being limited thereto, the compounds of Formula II-15, preferably, Formulae II-16 (Salinosporamide A), II-17, II-18 and II-19, can be obtained synthetically or by fermentation. Exemplary fermentation procedures are provided below. Further, the compounds of structure II-15, preferably, Formulae II-16, II-17, II-18 and II-19 can be used as starting compounds in order to obtain/synthesize various of the other compounds described herein. Exemplary non-limiting syntheses are provided herein.



II-16



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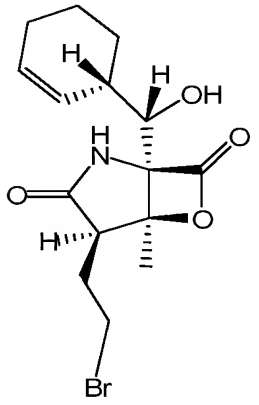
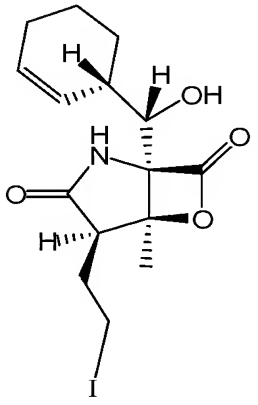


II-18



II-19

[0302] The compound of Formula II-16 may be produced through a high-yield saline fermentation (~350 - 400 mg/L) and modifications of the conditions have yielded new analogs in the fermentation extracts. Additional analogs can be generated through directed biosynthesis. Directed biosynthesis is the modification of a natural product by adding biosynthetic precursor analogs to the fermentation of producing microorganisms (Lam, *et al.*,

J Antibiot (Tokyo) 44:934 (1991), Lam, *et al.*, *J Antibiot (Tokyo)* 54:1 (2001); which is hereby incorporated by reference in its entirety).

[0303] Exposing the producing culture to analogs of acetic acid, phenylalanine, valine, butyric acid, shikimic acid, and halogens, preferably, other than chlorine, can lead to the formation of new analogs. The new analogs produced can be easily detected in crude extracts by HPLC and LC-MS. For example, after manipulating the medium with different concentrations of sodium bromide, a bromo-analog, the compound of Formula II-18, was successfully produced in shake-flask culture at a titer of 14 mg/L.

[0304] A second approach to generate analogs is through biotransformation. Biotransformation reactions are chemical reactions catalyzed by enzymes or whole cells containing these enzymes. Zaks, A., *Curr Opin Chem Biol* 5:130 (2001). Microbial natural products are ideal substrates for biotransformation reactions as they are synthesized by a series of enzymatic reactions inside microbial cells. Riva, S., *Curr Opin Chem Biol* 5:106 (2001).

[0305] Given the structure of the described compounds, including those of Formula I-15, for example, the possible biosynthetic origins are acetyl-CoA, ethylmalonyl-CoA, phenylalanine and chlorine. Ethylmalonyl-CoA is derived from butyryl-CoA, which can be derived either from valine or crotonyl-CoA. Liu, *et al.*, *Metab Eng* 3:40 (2001). Phenylalanine is derived from shikimic acid.

[0306] Alternatively, compounds such as structure II-16 and its analogs may be produced synthetically, e.g., such as described in United States Application Serial No. 11/697,689, which is incorporated by reference in its entirety.

Production of Compounds of Formulae I-7, II-16, II-17, II-18, II-20, II-24C, II-26, II-27 and II-28

[0307] The production of compounds of Formulae I-7, II-16, II-17, II-18, II-20,II-24C, II-26, II-27 and II-28 can be carried out by cultivating strain CNB476 and strain NPS21184, a natural variant of strain CNB476, in a suitable nutrient medium under conditions described herein, preferably under submerged aerobic conditions, until a substantial amount of compounds are detected in the fermentation; harvesting by extracting

the active components from the fermentation broth with a suitable solvent; concentrating the solvent containing the desired components; then subjecting the concentrated material to chromatographic separation to isolate the compounds from other metabolites also present in the cultivation medium.

[0308] The culture (CNB476) was deposited on June 20, 2003 with the American Type Culture Collection (ATCC) in Rockville, MD and assigned the ATCC patent deposition number PTA-5275. Strain NPS21184, a natural variant of strain CNB476, was derived from strain CNB476 as a single colony isolate. Strain NPS21184 has been deposited to ATCC on April 27, 2005. The ATCC deposit meets all of the requirements of the Budapest treaty. The culture is also maintained at and available from Nereus Pharmaceutical Culture Collection at 10480 Wateridge Circle, San Diego, CA 92121. In addition to the specific microorganism described herein, it should be understood that mutants, such as those produced by the use of chemical or physical mutagens including X-rays, etc. and organisms whose genetic makeup has been modified by molecular biology techniques, may also be cultivated to produce the starting compounds of Formulae II-16, II-17, and II-18.

Fermentation of strain CNB476 and strain NPS21184

[0309] Production of compounds can be achieved at temperature conducive to satisfactory growth of the producing organism, e.g. from 16°C to 40°C, but it is preferable to conduct the fermentation at 22°C to 32°C. The aqueous medium can be incubated for a period of time necessary to complete the production of compounds as monitored by high pressure liquid chromatography (HPLC), preferably for a period of about 2 to 10 days, on a rotary shaker operating at about 50 rpm to 400 rpm, preferably at 150 rpm to 250 rpm, for example. The production of the compounds can also be achieved by cultivating the production strain in a bioreactor, such as a fermentor system that is suitable for the growth of the production strain.

[0310] Growth of the microorganisms can be achieved by one of ordinary skill of the art by the use of appropriate medium. Broadly, the sources of carbon include glucose, fructose, mannose, maltose, galactose, mannitol and glycerol, other sugars and sugar alcohols, starches and other carbohydrates, or carbohydrate derivatives such as dextran,

cerelose, as well as complex nutrients such as oat flour, corn meal, millet, corn, and the like. The exact quantity of the carbon source that is utilized in the medium will depend in part, upon the other ingredients in the medium, but an amount of carbohydrate between 0.5 to 25 percent by weight of the medium can be satisfactorily used, for example. These carbon sources can be used individually or several such carbon sources can be combined in the same medium, for example. Certain carbon sources are preferred as hereinafter set forth.

[0311] The sources of nitrogen include amino acids such as glycine, arginine, threonine, methionine and the like, ammonium salt, as well as complex sources such as yeast extracts, corn steep liquors, distiller solubles, soybean meal, cotttonseed meal, fish meal, peptone, and the like. The various sources of nitrogen can be used alone or in combination in amounts ranging from 0.5 to 25 percent by weight of the medium, for example.

[0312] Among the nutrient inorganic salts, which can be incorporated in the culture media, are the customary salts capable of yielding sodium, potassium, magnesium, calcium, phosphate, sulfate, chloride, carbonate, and like ions. Also included are trace metals such as cobalt, manganese, iron, molybdenum, zinc, cadmium, and the like.

Pharmaceutical Compositions

[0313] In one embodiment, the compounds disclosed herein are used in pharmaceutical compositions. The compounds preferably can be produced by the methods disclosed herein. The compounds can be used, for example, in pharmaceutical compositions comprising a pharmaceutically acceptable carrier prepared for storage and subsequent administration. Also, embodiments relate to a pharmaceutically effective amount of the products and compounds disclosed above in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985), which is incorporated herein by reference in its entirety. Preservatives, stabilizers, dyes and even flavoring agents can be provided in the pharmaceutical composition. For example, sodium benzoate, ascorbic acid and esters of p-hydroxybenzoic acid can be added as preservatives. In addition, antioxidants and suspending agents can be used.

[0314] The compositions can be formulated and used as tablets, capsules, or elixirs for oral administration; suppositories for rectal administration; sterile solutions, suspensions for injectable administration; patches for transdermal administration, and sub-dermal deposits and the like. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride, and the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. If desired, absorption enhancing preparations (for example, liposomes), can be utilized.

[0315] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or other organic oils such as soybean, grapefruit or almond oils, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0316] Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are

provided with suitable coatings. For this purpose, concentrated sugar solutions can be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses. For this purpose, concentrated sugar solutions can be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses. Such formulations can be made using methods known in the art (see, for example, U.S. Patent Nos. 5,733,888 (injectable compositions); 5,726,181 (poorly water soluble compounds); 5,707,641 (therapeutically active proteins or peptides); 5,667,809 (lipophilic agents); 5,576,012 (solubilizing polymeric agents); 5,707,615 (anti-viral formulations); 5,683,676 (particulate medicaments); 5,654,286 (topical formulations); 5,688,529 (oral suspensions); 5,445,829 (extended release formulations); 5,653,987 (liquid formulations); 5,641,515 (controlled release formulations) and 5,601,845 (spheroid formulations); all of which are incorporated herein by reference in their entireties.

[0317] Further disclosed herein are various pharmaceutical compositions well known in the pharmaceutical art for uses that include topical, intraocular, intranasal, and intraauricular delivery. Pharmaceutical formulations include aqueous ophthalmic solutions of the active compounds in water-soluble form, such as eyedrops, or in gellan gum (Shedden et al., *Clin. Ther.*, 23(3):440-50 (2001)) or hydrogels (Mayer et al., *Ophthalmologica*, 210(2):101-3 (1996)); ophthalmic ointments; ophthalmic suspensions, such as microparticulates, drug-containing small polymeric particles that are suspended in a liquid carrier medium (Joshi, A. 1994 *J Ocul Pharmacol* **10**:29-45), lipid-soluble formulations (Alm et al., *Prog. Clin. Biol. Res.*, 312:447-58 (1989)), and microspheres (Mordenti, *Toxicol. Sci.*, 52(1):101-6 (1999)); and ocular inserts. All of the above-mentioned references, are incorporated herein by reference in their entireties. Such suitable pharmaceutical formulations are most often and preferably formulated to be sterile, isotonic and buffered for stability and comfort. Pharmaceutical compositions may also include drops and sprays often

prepared to simulate in many respects nasal secretions to ensure maintenance of normal ciliary action. As disclosed in Remington's Pharmaceutical Sciences (Mack Publishing, 18th Edition), which is incorporated herein by reference in its entirety, and well-known to those skilled in the art, suitable formulations are most often and preferably isotonic, slightly buffered to maintain a pH of 5.5 to 6.5, and most often and preferably include anti-microbial preservatives and appropriate drug stabilizers. Pharmaceutical formulations for intraauricular delivery include suspensions and ointments for topical application in the ear. Common solvents for such aural formulations include glycerin and water.

[0318] To formulate the compounds of Formulae I and II as an anti-cancer agent, known surface active agents, excipients, smoothing agents, suspension agents and pharmaceutically acceptable film-forming substances and coating assistants, and the like can be used. Preferably alcohols, esters, sulfated aliphatic alcohols, and the like can be used as surface active agents; sucrose, glucose, lactose, starch, crystallized cellulose, mannitol, light anhydrous silicate, magnesium aluminate, magnesium methasilicate aluminate, synthetic aluminum silicate, calcium carbonate, sodium acid carbonate, calcium hydrogen phosphate, calcium carboxymethyl cellulose, and the like can be used as excipients; magnesium stearate, talc, hardened oil and the like can be used as smoothing agents; coconut oil, olive oil, sesame oil, peanut oil, soya can be used as suspension agents or lubricants; cellulose acetate phthalate as a derivative of a carbohydrate such as cellulose or sugar, or methylacetate-methacrylate copolymer as a derivative of polyvinyl can be used as suspension agents; and plasticizers such as ester phthalates and the like can be used as suspension agents. In addition to the foregoing preferred ingredients, sweeteners, fragrances, colorants, preservatives and the like can be added to the administered formulation of the compound produced by the method of the embodiment, particularly when the compound is to be administered orally.

[0319] When used as an anti-cancer compound, for example, the compounds of Formulae I and II or compositions including compounds of Formulae I and II can be administered by either oral or non-oral pathways. When administered orally, it can be administered in capsule, tablet, granule, spray, syrup, or other such form. When administered non-orally, it can be administered as an aqueous suspension, an oily preparation or the like or

as a drip, suppository, salve, ointment or the like, when administered via injection, subcutaneously, intraperitoneally, intravenously, intramuscularly, or the like.

[0320] In one embodiment, the anti-cancer agent can be mixed with additional substances to enhance their effectiveness.

Methods of Administration

[0321] In an alternative embodiment, the disclosed chemical compounds and the disclosed pharmaceutical compositions are administered by a particular method as an anti-cancer, anti-microbial or anti-inflammatory. Such methods include, among others, (a) administration though oral pathways, which administration includes administration in capsule, tablet, granule, spray, syrup, or other such forms; (b) administration through non-oral pathways, which administration includes administration as an aqueous suspension, an oily preparation or the like or as a drip, suppository, salve, ointment or the like; administration via injection, subcutaneously, intraperitoneally, intravenously, intramuscularly, intradermally, or the like; as well as (c) administration topically, (d) administration rectally, or (e) administration vaginally, as deemed appropriate by those of skill in the art for bringing the compound of the present embodiment into contact with living tissue; and (f) administration via controlled released formulations, depot formulations, and infusion pump delivery. As further examples of such modes of administration and as further disclosure of modes of administration, disclosed herein are various methods for administration of the disclosed chemical compounds and pharmaceutical compositions including modes of administration through intraocular, intranasal, and intraauricular pathways.

[0322] The pharmaceutically effective amount of the compositions that include the described compounds required as a dose will depend on the route of administration, the type of animal, including human, being treated, and the physical characteristics of the specific animal under consideration. The dose can be tailored to achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize. In a typical embodiment, a compound represented by Formulae I and II can be administered to a patient in need of an anti-cancer agent, until the need is effectively reduced or preferably removed.

[0323] In practicing the methods of the embodiment, the products or compositions can be used alone or in combination with one another, or in combination with other therapeutic or diagnostic agents. These products can be utilized *in vivo*, ordinarily in a mammal, preferably in a human, or *in vitro*. In employing them *in vivo*, the products or compositions can be administered to the mammal in a variety of ways, including parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, vaginally, nasally or intraperitoneally, employing a variety of dosage forms. Such methods may also be applied to testing chemical activity *in vivo*.

[0324] As will be readily apparent to one skilled in the art, the useful *in vivo* dosage to be administered and the particular mode of administration will vary depending upon the age, weight and mammalian species treated, the particular compounds employed, and the specific use for which these compounds are employed. The determination of effective dosage levels, that is the dosage levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine pharmacological methods. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable *in vitro* studies can be used to establish useful doses and routes of administration of the compositions identified by the present methods using established pharmacological methods.

[0325] In non-human animal studies, applications of potential products are commenced at higher dosage levels, with dosage being decreased until the desired effect is no longer achieved or adverse side effects disappear. The dosage may range broadly, depending upon the desired affects and the therapeutic indication. Typically, dosages can be between about 10 mg/kg and 100 mg/kg body weight, preferably between about 100 mg/kg and 10 mg/kg body weight. Alternatively dosages can be based and calculated upon the surface area of the patient, as understood by those of skill in the art. Administration is preferably oral on a daily or twice daily basis.

[0326] The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. *See* for example, Fingl *et al.*, in The Pharmacological Basis of Therapeutics, 1975, which is incorporated herein by reference in its entirety. It should be noted that the attending physician would know how to and when

to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above can be used in veterinary medicine.

[0327] Depending on the specific conditions being treated, such agents can be formulated and administered systemically or locally. A variety of techniques for formulation and administration can be found in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990), which is incorporated herein by reference in its entirety. Suitable administration routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

[0328] For injection, the agents of the embodiment can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the embodiment into dosages suitable for systemic administration is within the scope of the embodiment. With proper choice of carrier and suitable manufacturing practice, the compositions disclosed herein, in particular, those formulated as solutions, can be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the embodiment to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

[0329] Agents intended to be administered intracellularly can be administered using techniques well known to those of ordinary skill in the art. For example, such agents can be encapsulated into liposomes, then administered as described above. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external micro-environment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules can be directly administered intracellularly.

[0330] Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration can be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions can be manufactured in a manner that is itself known, for example, by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

[0331] Compounds disclosed herein can be evaluated for efficacy and toxicity using known methods. For example, the toxicology of a particular compound, or of a subset of the compounds, sharing certain chemical moieties, can be established by determining *in vitro* toxicity towards a cell line, such as a mammalian, and preferably human, cell line. The results of such studies are often predictive of toxicity in animals, such as mammals, or more specifically, humans. Alternatively, the toxicity of particular compounds in an animal model, such as mice, rats, rabbits, dogs or monkeys, can be determined using known methods. The efficacy of a particular compound can be established using several art recognized methods, such as *in vitro* methods, animal models, or human clinical trials. Art-recognized *in vitro* models exist for nearly every class of condition, including the conditions abated by the compounds disclosed herein, including cancer, cardiovascular disease, and various immune dysfunction, and infectious diseases. Similarly, acceptable animal models can be used to establish efficacy of chemicals to treat such conditions. When selecting a model to determine

efficacy, the skilled artisan can be guided by the state of the art to choose an appropriate model, dose, and route of administration, and regime. Of course, human clinical trials can also be used to determine the efficacy of a compound in humans.

[0332] In the case of using a compound produced by methods of the embodiment as a biochemical test reagent, the compound produced by methods of the embodiment inhibits the progression of the disease when it is dissolved in an organic solvent or hydrous organic solvent and it is directly applied to any of various cultured cell systems. Usable organic solvents include, for example, methanol, methylsulfoxide, and the like. The formulation can, for example, be a powder, granular or other solid inhibitor, or a liquid inhibitor prepared using an organic solvent or a hydrous organic solvent. While a preferred concentration of the compound produced by the method of the embodiment for use as an anticancer compound is generally in the range of about 1 to about 100 μg/mL, the most appropriate use amount varies depending on the type of cultured cell system and the purpose of use, as will be appreciated by persons of ordinary skill in the art. Also, in certain applications it can be necessary or preferred to persons of ordinary skill in the art to use an amount outside the foregoing range.

[0333] As will be understood by one of skill in the art, "need" is not an absolute term and merely implies that the patient can benefit from the treatment of the anti-cancer agent in use. By "patient" what is meant is an organism that can benefit by the use of an anti-cancer agent. For example, any organism with cancer, such as, pancreatic cancer. In one embodiment, the patient's health may not require that an anti-cancer agent be administered, however, the patient may still obtain some benefit by the reduction of the level of cancer cells present in the patient, and thus be in need. In one embodiment, the anti- anti-cancer agent is effective against one type of cancer, but not against other types; thus, allowing a high degree of selectivity in the treatment of the patient. In choosing such an anti-cancer agent, the methods and results disclosed in the Examples can be useful. In still further embodiments, the anti-cancer agent is effective against a broad spectrum of cancers or all cancers. Examples of cancers, against which the compounds can be effective include pancreatic cancer, a colorectal carcinoma, a prostate carcinoma, a breast adenocarcinoma, a non-small cell lung carcinoma, an ovarian carcinoma, multiple myelomas, a melanoma, and the like.

[0334] "Therapeutically effective amount," "pharmaceutically effective amount," or similar term, means that amount of drug or pharmaceutical agent that will result in a biological or medical response of a cell, tissue, system, animal, or human that is being sought. In a preferred embodiment, the medical response is one sought by a researcher, veterinarian, medical doctor, or other clinician.

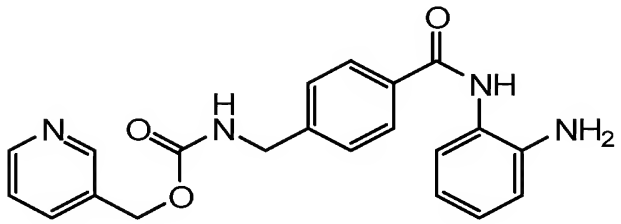
[0335] "Anti-cancer agent" refers to a compound or composition including the compound that reduces the likelihood of survival of a cancer cell. In one embodiment, the likelihood of survival is determined as a function of an individual cancer cell; thus, the anti-cancer agent will increase the chance that an individual cancer cell will die. In one embodiment, the likelihood of survival is determined as a function of a population of cancer cells; thus, the anti-cancer agent will increase the chances that there will be a decrease in the population of cancer cells. In one embodiment, anti-cancer agent means chemotherapeutic agent or other similar term.

[0336] A "chemotherapeutic agent" is a chemical compound useful in the treatment of a neoplastic disease, such as cancer. Examples of chemotherapeutic agents include alkylating agents, such as a nitrogen mustard, an ethyleneimine and a methylmelamine, an alkyl sulfonate, a nitrosourea, and a triazene, folic acid antagonists, anti-metabolites of nucleic acid metabolism, antibiotics, pyrimidine analogs, 5-fluorouracil, cisplatin, purine nucleosides, amines, amino acids, triazol nucleosides, corticosteroids, a natural product such as a vinca alkaloid, an epipodophyllotoxin, an antibiotic, an enzyme, a taxane, and a biological response modifier or antibodies to biological response modifiers or other agents; miscellaneous agents such as a platinum coordination complex, an anthracenedione, an anthracycline, a substituted urea, a methyl hydrazine derivative, or an adrenocortical suppressant; or a hormone or an antagonist such as an adrenocorticosteroid, a progestin, an estrogen, an antiestrogen, an androgen, an antiandrogen, or a gouadotropin-releasing hormone analog. Specific examples include Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiotepa, Busulfan, Cytoxin, Taxol, Toxotere, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincreistine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins,

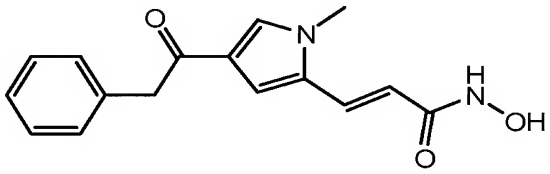
Esperamicins, Melphalan, and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors, such as tamoxifen and onapristone.

[0337] Additional examples of such chemotherapeutics include alkaloids, alkylating agents, antibiotics, antimetabolites, enzymes, hormones, platinum compounds, immunotherapeutics (antibodies, T-cells, epitopes), BRMs, and the like. Examples include, Vincristine, Vinblastine, Vindesine, Paclitaxel (Taxol), Docetaxel, topoisomerase inhibibitors epipodophyllotoxins (Etoposide (VP-16), Camptothecin, nitrogen mustards (cyclophosphamide Cytoxan), Nitrosoureas, Carmustine, lomustine, dacarbazine, hydroxymethylmelamine, thiotepa and mitocycin C, Dactinomycin (Actinomycin D), anthracycline antibiotics (Daunorubicin, Daunomycin, Cerubidine), Doxorubicin (Adriamycin), Idarubicin (Idamycin), Anthracenediones (Mitoxantrone), Bleomycin (Blenoxane), Plicamycin (Mithramycin, Antifolates (Methotrexate (Folex, Mexate)), purine antimetabolites (6-mercaptopurine (6-MP, Purinethol) and 6- thioguanine (6-TG). The two major anticancer drugs in this category are 6-mercaptopurine and 6-thioguanine, Chlorodeoxyadenosine and Pentostatin, Pentostatin (2'-deoxycoformycin), pyrimidine antagonists, Avastin, Leucovorin, Oxaliplatin, fluoropyrimidines (5-fluorouracil(Adrucil), 5-fluorodeoxyuridine (FdUrd) (Floxuridine)), Cytosine Arabinoside (Cytosar, ara-C), Fludarabine, L-asparaginase, Hydroxyurea, glucocorticoids, antiestrogens, tamoxifen, nonsteroidal antiandrogens, flutamide, aromatase inhibitors Anastrozole(Arimidex), Cisplatin, 6-Mercaptopurine and Thioguanine, Methotrexate, Cytoxan, Cytarabine, L-Asparaginase, Steroids: Prednisone and Dexamethasone, bevacizumab, and gemcitabine. Also, proteasome inhibitors such as bortezomib and carfilzomib (PR-171) can be used in combination with the instant compounds, for example. Examples of biologics can include agents such as TRAIL, antibodies to TRAIL and agonistic antibodies TRAIL death receptors DR4 and DR5, integrins such as alpha-V-beta-3 ($\alpha V\beta 3$) and / or other cytokine/growth factors that are involved in angiogenesis, VEGF, EGF, FGF and PDGF and antibodies to these cytokines/growth factors such as Erbitux. In some aspects, the compounds can be conjugated to or delivered with an antibody.

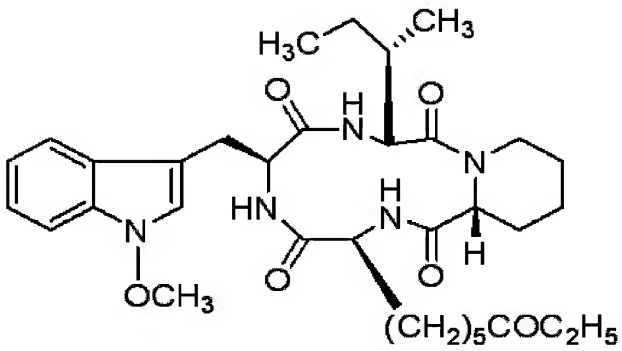
[0338] In some embodiments, the additional chemotherapeutic is a histone deacetylase inhibitor (HDACi). In various embodiments, the HDACi is selected from the group consisting of:



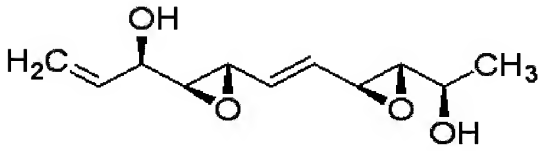
(pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275 or SNDX-275),



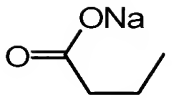
APHA compound 8,



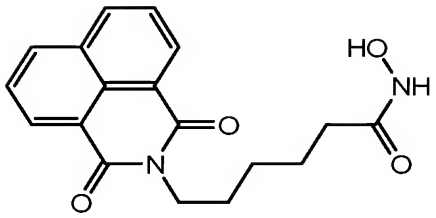
apicidin,



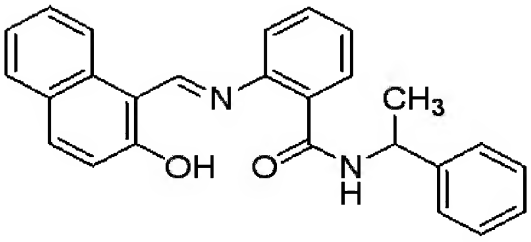
(-)-Depudecin,



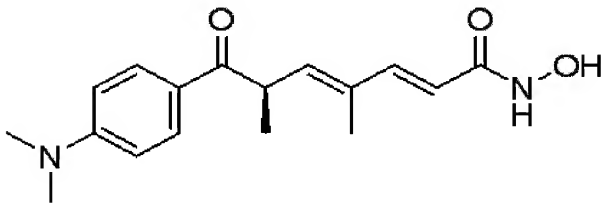
sodium Butyrate,



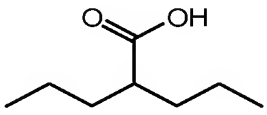
Scriptaid,



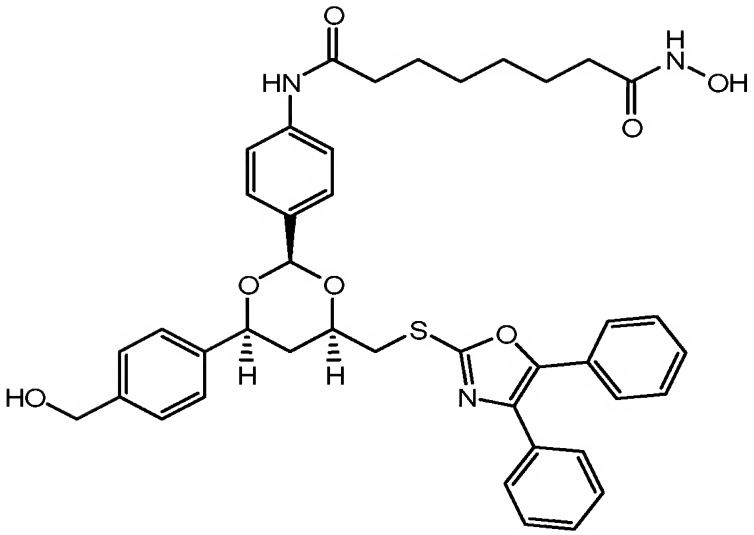
Sirtinol,



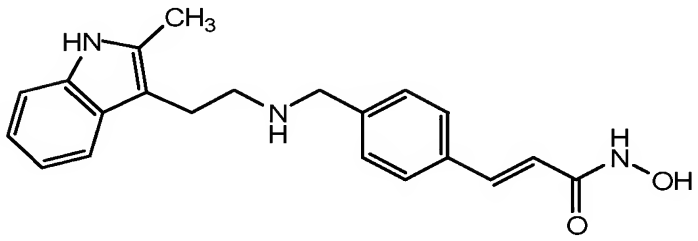
trichostatin A,



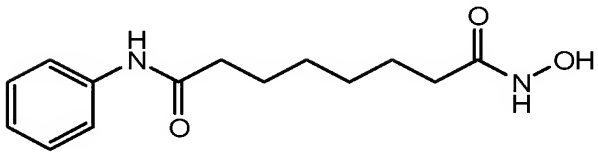
valproic acid,



tubacin,

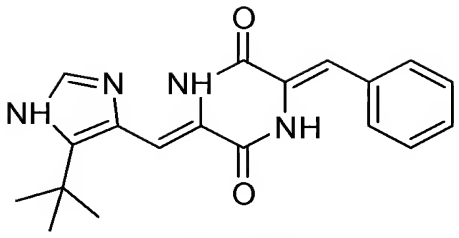


panobinostat, and



vorinostat (suberoylanilide hydroxamic acid (SAHA)).

[0339] In other embodiments, the additional chemotherapeutic is a vascular disrupting agents (VDA). Examples of such VDAs include combratostatin CA4P and NPI-2358. NPI-2358 is represented by the following formula:



NPI-2358

[0340] The anti-cancer agent may act directly upon a cancer cell to kill the cell, induce death of the cell, to prevent division of the cell, and the like. Alternatively, the anti-cancer agent may indirectly act upon the cancer cell by limiting nutrient or blood supply to the cell, for example. Such anti-cancer agents are capable of destroying or suppressing the growth or reproduction of cancer cells, such as a colorectal carcinoma, a prostate carcinoma, a breast adenocarcinoma, a non-small cell lung carcinoma, an ovarian carcinoma, multiple myelomas, a melanoma, and the like.

[0341] In one embodiment, a described compound, preferably a compound having the Formula I, including those as described herein, is considered an effective anti-cancer agent if the compound can influence 10% of the cancer cells, for example. In a more preferred embodiment, the compound is effective if it can influence 10 to 50% of the cancer cells. In an even more preferred embodiment, the compound is effective if it can influence 50-80% of the cancer cells. In an even more preferred embodiment, the compound is effective if it can influence 80-95% of the cancer cells. In an even more preferred embodiment, the compound is effective if it can influence 95-99% of the cancer cells. "Influence" is defined by the mechanism of action for each compound. For example, if a compound prevents the division of cancer cells, then influence is a measure of prevention of cancer cell division. Not all mechanisms of action need be at the same percentage of effectiveness. In an alternative embodiment, a low percentage effectiveness can be desirable if the lower degree of effectiveness is offset by other factors, such as the specificity of the compound, for example. Thus a compound that is only 10% effective, for example, but displays little in the way of harmful side-effects to the host, or non-harmful microbes or cells, can still be considered effective.

[0342] In one embodiment, the compounds described herein are administered simply to remove cancer cells and need not be administered to a patient. For example, the compounds can be administered *ex vivo* to a cell sample, such as a bone marrow or stem cell transplant to ensure that only non-cancerous cells are introduced into the recipient. After the compounds are administered they may optionally be removed. Whether or not this is an option will depend upon the relative needs of the situation and the risks associated with the compound, which in part can be determined as described in the Examples below.

[0343] The following non-limiting examples are meant to describe the preferred embodiments of the methods. Variations in the details of the particular methods employed and in the precise chemical compositions obtained will undoubtedly be appreciated by those of skill in the art.

EXAMPLES

EXAMPLE 1

FERMENTATION OF COMPOUND OF FORMULAE I-7, II-16, II-17, II-20, II-24C, II-26 AND II-28 USING STRAIN CNB476

[0344] Strain CNB476 was grown in a 500-mL flask containing 100 mL of vegetative medium consisting of the following per liter of deionized water: glucose, 4 g; Bacto tryptone, 3 g; Bacto casitone, 5 g; and synthetic sea salt (Instant Ocean, Aquarium Systems), 30 g. The first seed culture was incubated at 28°C for 3 days on a rotary shaker operating at 250 rpm. Five mL each of the first seed culture was inoculated into three 500-ml flasks containing of 100 mL of the vegetative medium. The second seed cultures were incubated at 28°C and 250 rpm on a rotary shaker for 2 days. Five mL each of the second seed culture was inoculated into thirty-five 500-mL flasks containing of 100 mL of the vegetative medium. The third seed cultures were incubated at 28°C and 250 rpm on a rotary shaker for 2 days. Five mL each of the third seed culture was inoculated into four hundred 500-mL flasks containing 100 mL of the Production Medium A consisting of the following per liter of deionized water: starch, 10 g; yeast extract, 4 g; Hy-Soy, 4 g; ferric sulfate, 40 mg; potassium bromide, 100 mg; calcium carbonate, 1 g; and synthetic sea salt (Instant Ocean, Aquarium Systems), 30 g. The production cultures were incubated at 28°C and 250 rpm on

roatry shakers for 1 day. Approximately 2 to 3 grams of sterile Amberlite XAD-7 resin were added to the production cultures. The production cultures were further incubated at 28°C and 250 rpm on rotary shakers for 5 days and achieved a titer of Compound II-16 of about 200 mg/L. The culture broth was filtered through cheese cloth to recover the Amberlite XAD-7 resin. The resin was extracted with 2 times 6 liters ethyl acetate followed by 1 time 1.5 liters ethyl acetate. The combined extracts were dried in vacuo. The dried extract, containing 3.8 grams the compound of Formula II-16 and lesser quantities of compounds of formulae II-20 and II-24C, was then processed for the recovery of the compounds of Formula I-7, II-16, II-20, II-24C, II-26 and II-28.

EXAMPLE 2

FERMENTATION OF COMPOUNDS I-7, II-16, II-17, II-20, II-24C, II-26 AND II-28 USING STRAIN NPS21184

[0345] Strain NPS21184 was grown in a 500-mL flask containing 100 mL of vegetative medium consisting of the following per liter of deionized water: glucose, 8 g; yeast extract, 6 g; Hy-Soy, 6 g; and synthetic sea salt (Instant Ocean, Aquarium Systems), 30 g. The first seed culture was incubated at 28°C for 3 days on a rotary shaker operating at 250 rpm. Five mL of the first seed culture was inoculated into 500-mL flask containing of 100 mL of the vegetative medium. The second seed cultures were incubated at 28°C and 250 rpm on a rotary shaker for 2 days. Five mL each of the second seed culture was inoculated into 500-mL flask containing of 100 mL of the vegetative medium. The third seed cultures were incubated at 28°C and 250 rpm on a rotary shaker for 2 days. Five mL each of the third seed culture was inoculated into 500-mL flask containing 100 mL of the Production Medium B consisting of the following per liter of deionized water: starch, 20 g; yeast extract, 4 g; Hy-Soy, 8 g; ferric sulfate, 40 mg; potassium bromide, 100 mg; calcium carbonate, 1 g; and synthetic sea salt (Instant Ocean, Aquarium Systems), 30 g. The production cultures were incubated at 28°C and 250 rpm on rotary shakers for 1 day. Approximately 2 to 3 grams of sterile Amberlite XAD-7 resin were added to the production culture. The production culture was further incubated at 28°C and 250 rpm on rotary shaker for 4 days and achieved a titer of 350 – 400 mg/L for Compound II-16.

[0346] Alternatively, the production of the compounds can be achieved in a 42L fermentor system using strain NPS21184. Strain NPS21184 was grown in a 500-mL flask containing 100 mL of vegetative medium consisting of the following per liter of deionized water: glucose, 8 g; yeast extract, 6 g; Hy-Soy, 6 g; and synthetic sea salt (Instant Ocean, Aquarium Systems), 30 g. The first seed culture was incubated at 28°C for 3 days on a rotary shaker operating at 250 rpm. Five mL of the first seed culture was inoculated into 500-mL flask containing of 100 mL of the vegetative medium. The second seed cultures were incubated at 28°C and 250 rpm on a rotary shaker for 2 days. Twenty mL each of the second seed culture was inoculated into 2.8 L Fernbach flask containing of 400 mL of the vegetative medium. The third seed cultures were incubated at 28°Cand 250 rpm on a rotary shaker for 2 days. 1.2 L of the third seed culture was inoculated into a 42 L fermentor containing 26 L of Production Medium A. Production Medium B and Production Medium C, with the following composition, can also be used. Production Medium C consisting of the following per liter of deionized water: starch, 15 g; yeast extract 6 g; Hy-Soy, 6 g; ferric sulfate, 40 mg; potassium bromide, 100 mg; calcium carbonate, 1 g; and synthetic sea salt (Instant Ocean, Aquarium Systems), 30 g. The fermentor cultures were operated at the following parameters: temperature, 28°C; agitation, 200 rpm; aeration, 13 L/min and back pressure, 4.5 psi. At 36 to 44 hours of the production cycle, approximately 600 grams of sterile Amberlite XAD-7 resin were added to the fermentor culture. The production culture was further incubated at the above operating parameters until day 4 of the production cycle. The aeration rate was lowered to 8 L/min. At day 5 of the production cycle, the fermentor culture achieved a titer of about 300 mg/L for Compound II-16. The culture broth was filtered through cheese cloth to recover the Amberlite XAD-7 resin. The resin was extracted with 2 times 4.5 liters ethyl acetate followed by 1 time 1.5 liters ethyl acetate. The combined extracts were dried in vacuo. The dried extract was then processed for the recovery of the Compounds of Formulae I-7, II-16, II-17, II-20, II-24C, II-26 and II-28.

EXAMPLE 3

PURIFICATION OF COMPOUND OF FORMULAE I-7, II-16, II-20, II-24C, II-26 AND II-28

3A: Purification of Compound of Formulae II-16, II-20, II-24C, II-26 and II-28

[0347] The pure compounds of Formulae II-16, II-20 II-24C, II-26 and II-28 were obtained by flash chromatography followed by HPLC. Eight grams crude extract containing 3.8 grams of the compound of Formula II-16 and lesser quantities of II-20, II-24C, II-26 and II-28 was processed by flash chromatography using Biotage Flash40i system and Flash 40M cartridge (KP-Sil Silica, 32-63 μm, 90 grams). The flash chromatography was developed by the following step gradient:

1. Hexane (1L)
2. 10% Ethyl acetate in hexane (1 L)
3. 20% Ethyl acetate in hexane, first elution (1 L)
4. 20% Ethyl acetate in hexane, second elution (1 L)
5. 20% Ethyl acetate in hexane, third elution (1 L)
6. 25% Ethyl acetate in hexane (1 L)
7. 50% Ethyl acetate in hexane (1 L)
8. Ethyl acetate (1 L)

[0348] Fractions containing the compound of Formula II-16 in greater or equal to 70% UV purity by HPLC were pooled and subject to HPLC purification, as described below, to obtain II-16, along with II-20 and II-24C, each as pure compounds

Column	Phenomenex Luna 10 μm Silica
Dimensions	25 cm X 21.2 mm ID
Flow rate	25 mL/min
Detection	ELSD
Solvent	Gradient of 24% EtOAc/hexane for 19 min, 24% EtOAc/hexane to 100%EtOAc in 1 min, then 100% EtOAc for 4 min

[0349] The fraction enriched in compound of Formula II-16 (described above; ~ 70% pure with respect to II-16) was dissolved in acetone (60 mg/mL). Aliquots (950 μL) of this solution were injected onto a normal-phase HPLC column using the conditions described above. Compound II-16 typically eluted after 14 minutes and compounds II-24C and II-26 co-eluted as a single peak at 11 min. When parent samples containing compounds II-17, II-20 and II-28 were processed, compound II-17 eluted at 22 minutes, while II-20 and II-28 co-eluted at 23 minutes during the 100% ethyl acetate wash. Fractions containing compound II-16 and minor analogs were pooled based on composition of compounds present, and evaporated under reduced pressure on a rotary evaporator. This process yielded pure Compound A, as well as separate fractions containing minor compounds II-20, II-24C, II-26 and II-28, which were further purified as described below.

[0350] Sample containing II-24C and II-26 generated from the process described above were further separated using reversed-phase preparative HPLC as follows. The sample containing II-24C (70 mg) was dissolved in acetonitrile at a concentration of 10 mg/mL, and 500 μL was loaded on an HPLC column of dimensions 21 mm i.d. by 15 cm length containing Eclipse XDB-C18 support. The solvent gradient increased linearly from 15% acetonitrile /85% water to 100% acetonitrile over 23 minutes at a flow rate of 14.5 mL/min. The solvent composition was held at 100% acetonitrile for 3 minutes before returning to the starting solvent mixture. Compound II-26 eluted at 17.5 minutes while compound II-24C eluted at 19 minutes under these conditions.

[0351] Crystalline II-26 was obtained using a vapor diffusion method. Compound II-26 (15 mg) was dissolved in 100 μL of acetone in a 1.5 mL v-bottom HPLC vial. This vial was then placed inside a larger sealed vessel containing 1 mL of pentane. Crystals suitable for X-ray crystallography experiments were observed along the sides and bottom of the inner vial after 48 hours of incubation at 4°C. Crystallography data was collected on a Bruker SMART APEX CCD X-ray diffractometer (F(000)= 2656, $Mo_{K\alpha}$ radiation, λ=0.71073 Å, μ=0.264 mm^{-1}, T=100K) at the UCSD Crystallography Lab and the refinement method used was full-matrix least-squares on F^2. Crystal data NPI-2065: $C_{15}H_{20}ClNO_4$, MW=313.77, tetragonal, space group P4(1)2(1)2, a= b=11.4901(3) Å, c= 46.444(2) Å, $\alpha=\beta=\gamma=90°$,

vol=6131.6(3) $Å^3$, Z=16, ρ_{calcd}=1.360 g cm^{-3}, crystal size, 0.30 x 0.15 x 0.07 mm^3, θ range, 1.75-26.00°, 35367 reflections collected, 6025 independent reflections (R_{int}=0.0480), final R indices *(I>2σ(I))*: R_1=0.0369, wR_2=0.0794, GOF=1.060.

[0352] In order to separate II-28 from II-20, a reverse-phase isocratic method was employed. Sample (69.2 mg) containing both compounds was dissolved in acetonitrile to a concentration of 10 mg/mL, and 500 μL was loaded on a reverse-phase HPLC column (ACE 5 C18-HL, 15 cm X 21 mm ID) per injection. An isocratic solvent system of 27% acetonitrile/ 63% water at flow rate of 14.5 mL/min was used to separate compounds II-28 and II-20, which eluted after 14 and 16 minutes, respectively. Fractions containing compounds of interest were immediately evaporated under reduced pressure at room temperature on a rotary evaporator. Samples were then loaded onto a small column of silica and eluted with 10 mL of 70% hexane/30% acetone to remove additional impurities.

[0353] Samples generated from the preparative normal-phase HPLC method described above that contained II-20, but which were free of II-28 could also be triturated with 100% EtOAc to remove minor lipophilic impurities.

[0354] Compound of Formula II-16: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm. Low Res. Mass: m/z 314 (M+H), 336 (M+Na).

[0355] Compound of Formula II-20: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm. Low Res. Mass: m/z 266 (M+H); HRMS (ESI), *m/z* 266.1396 (M+H), Δ_{calc}= 1.2 ppm.

[0356] Compound of Formula II-24C: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm. Low Res. Mass: m/z 328 (M+H), 350 (M+Na); HRMS (ESI), *m/z* 328.1309 (M+H), Δ_{calc}= -2.0 ppm, $C_{16}H_{23}NO_4Cl$.

[0357] Compound of Formula II-26: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm; HRMS (ESI), *m/z* 314.1158 (M+H), Δ_{calc}= -0.4 ppm, $C_{15}H_{21}NO_4Cl$.

[0358] Compound of Formula II-28: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm; HRMS (ESI), *m/z* 266.1388 (M+H), Δ_{calc}= -1.8 ppm, $C_{14}H_{20}NO_4$.

3B: Purification of Compound of Formula I-7

[0359] A Biotage Flash 75Li system with a Flash 75L KP-Sil cartridge was used to process the filtered crude extract (10.0 g), enriched in Compound II-16 and containing

Compound of Formula I-7. The crude extract was dissolved to a concentration of 107 mg/mL in acetone and loaded directly onto the cartridge. The following solvent step gradient was then run through the cartridge at a flow rate between 235 mL/min and 250 mL/min

1. 10% EtOAc in n-Heptane (3.2 L)
2. 25% EtOAc in n-Heptane (16 L)
3. 30% EtOAc in n-Heptane (5.4 L)

[0360] Fractions enriched in Compound II-16 were pooled and concentrated by rotavapor until ~ 5% of the total pooled volume of solvent remained. The solvent was removed, leaving behind the white solid.

[0361] A crystallization was then performed on the solid by dissolving the sample (4.56 g) in 1:1 acetone:n-heptane (910 mL). The solvent was slowly evaporated using a rotary evaporator until the solvent was reduced to about 43% of its original volume. The solution (supernatant) was removed and concentrated (598 mg).

[0362] The supernatant was dissolved in acetone (80 mg/mL). Aliquots (500 μL) of this solution were injected onto a normal-phase HPLC column using the conditions described above for normal phase purification of Compounds II-16, II-24C, II-26 and II-28. Compound of Formula I-7 eluted at 7.5 minutes as a pure compound.

[0363] Compound of Formula I-7 (FIG. 58): UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm. Low Res. Mass: m/z 298 (M+H), 320 (M+Na).

EXAMPLE 4

FERMENTATION OF COMPOUNDS OF FORMULAE II-17, II-18, AND II-27

[0364] Strain CNB476 was grown in a 500-mL flask containing 100 mL of the first vegetative medium consisting of the following per liter of deionized water: glucose, 4 g; Bacto tryptone, 3 g; Bacto casitone, 5 g; and synthetic sea salt (Instant Ocean, Aquarium Systems), 30 g. The first seed culture was incubated at 28 C for 3 days on a rotary shaker operating at 250 rpm. Five mL of the first seed culture was inoculated into a 500-mL flask containing 100 mL of the second vegetative medium consisting of the following per liter of deionized water: starch, 10 g; yeast extract, 4 g; peptone, 2 g; ferric sulfate, 40 mg; potassium bromide, 100 mg; calcium carbonate, 1 g; and sodium bromide, 30 g. The second seed

cultures were incubated at 28°C for 7 days on a rotary shaker operating at 250 rpm. Approximately 2 to 3 gram of sterile Amberlite XAD-7 resin were added to the second seed culture. The second seed culture was further incubated at 28°C for 2 days on a rotary shaker operating at 250 rpm. Five ml of the second seed culture was inoculated into a 500-ml flask containing 100 mL of the second vegetative medium. The third seed culture was incubated at 28°C for 1 day on a rotary shaker operating at 250 rpm. Approximately 2 to 3 gram of sterile Amberlite XAD-7 resin were added to the third seed culture. The third seed culture was further incubated at 28°C for 2 days on a rotary shaker operating at 250 rpm. Five ml of the third culture was inoculated into a 500-ml flask containing 100 mL of the second vegetative medium. The fourth seed culture was incubated at 28°C for 1 day on a rotary shaker operating at 250 rpm. Approximately 2 to 3 gram of sterile Amberlite XAD-7 resin were added to the fourth seed culture. The fourth seed culture was further incubated at 28°C for 1 day on a rotary shaker operating at 250 rpm. Five mL each of the fourth seed culture was inoculated into ten 500-mL flasks containing 100 mL of the second vegetative medium. The fifth seed cultures were incubated at 28°C for 1 day on a rotary shaker operating at 250 rpm. Approximately 2 to 3 grams of sterile Amberlite XAD-7 resin were added to the fifth seed cultures. The fifth seed cultures were further incubated at 28°C for 3 days on a rotary shaker operating at 250 rpm. Four mL each of the fifth seed culture was inoculated into one hundred and fifty 500-mL flasks containing 100 mL of the production medium having the same composition as the second vegetative medium. Approximately 2 to 3 grams of sterile Amberlite XAD-7 resin were also added to the production culture. The production cultures were incubated at 28°C for 6 day on a rotary shaker operating at 250 rpm. The culture broth was filtered through cheese cloth to recover the Amberlite XAD-7 resin. The resin was extracted with 2 times 3 liters ethyl acetate followed by 1 time 1 liter ethyl acetate. The combined extracts were dried *in vacuo*. The dried extract, containing 0.42 g of the compound Formula II-17 and 0.16 gram the compound of Formula II-18, was then processed for the recovery of the compounds.

EXAMPLE 5

PURIFICATION OF COMPOUNDS OF FORMULA II-17, II-18 AND II-27

[0365] The pure compounds of Formula II-17 and II-18 were obtained by reversed-phase HPLC as described below:

Column	ACE 5 C18-HL
Dimensions	15 cm X 21 mm ID
Flow rate	14.5 mL/min
Detection	214 nm
Solvent	Gradient of 35% acetonitrile/65% H_2O to 90% acetonitrile/10% H_2O over 15 min

[0366] Crude extract (100 mg) was dissolved in 15 mL of acetonitrile. Aliquots (900 μL) of this solution were injected onto a reversed-phase HPLC column using the conditions described above. Compounds of Formulae II-17 and II-18 eluted at 7.5 and 9 minutes, respectively. Fractions containing the pure compounds were first concentrated using nitrogen to remove organic solvent. The remaining solution was then frozen and lyophilized to dryness.

[0367] An alternative purification method for Compound II-17 and II-18 was developed for larger scale purification and involved fractionation of the crude extract on a normal phase VLC column. Under these conditions, sufficient amounts of several minor metabolites were identified, including compound II-27. The crude extract (2.4 g) was dissolved in acetone (10 mL) and this solution adsorbed onto silica gel (10 cc) by drying *in vacuo*. The adsorbed crude extract was loaded on a normal phase silica VLC column (250 cc silica gel, column dimensions 2.5 cm diameter by 15 cm length) and washed with a step gradient of hexane / EtOAc, increasing in the percentage of hexane in steps of 5% (100 mL solvent per step). The majority of compound II-16 eluted in the 60% hexane / 40% EtOAc wash while the majority of compound II-17 eluted in the 50% hexane / 50% ethyl acetate wash. Final separation of the compounds was achieved using C18 HPLC chromatography (ACE 5 μm C18-HL, 150 mm X 21 mm ID) using an isocratic solvent system consisting of

35% acetonitrile/65% H_2O. Under these conditions, compound II-27 eluted at 11 minutes, compound II-17 eluted at 12.0 minutes, traces of compound A eluted at 23.5 minutes, and compound II-18 eluted at 25.5 minutes. The resulting samples were dried *in vacuo* using no heat to remove the aqueous solvent mixture. The spectroscopic data for these samples of compound II-16 and compound II-18 were found to be identical with those of samples prepared from earlier purification methods. The sample of compound II-18 was found to contain 8% of the lactone hydrolysis product and was further purified by washing through a normal phase silica plug (1 cm diameter by 2 cm height) and eluting using a solvent mixture of 20% EtOAc / 80% Hexanes (25 mL). The resulting sample was found to contain pure compound II-18.

[0368] The fractions containing compound II-27 described above were further purified using normal phase semipreparative HPLC (Phenomenex Luna Si 10 μm, 100 Å; 250 x 10 mm id) using a solvent gradient increasing from 100% hexane to 100% EtOAc over 20 minutes with a flowrate of 4 mL/min. Compound II-27 eluted as a pure compound after 11.5 minutes (0.8 mg, 0.03% isolated yield from dried extract weight).

[0369] Compound of Formula II-17: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm. High Res. Mass (APCI): m/z 280.156 (M+H), Δ_{calc}=2.2 ppm, $C_{15}H_{22}NO_4$.

[0370] Compound of Formula II-18: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm. High Res. Mass (APCI): m/z 358.065 (M+H), Δ_{calc}= -1.9 ppm, $C_{15}H_{21}NO_4Br$.

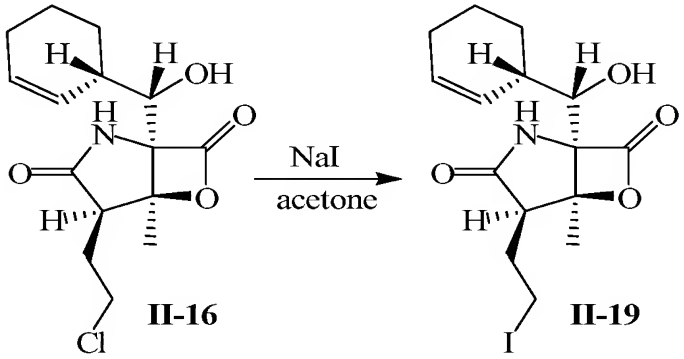
[0371] Compound II-27: UV (Acetonitrile/H_2O) λ_{max} 225(sh) nm; MS (HR-ESI), *m/z* 280.1556 (M+H) Δ_{calc}= 2.7 ppm ($C_{15}H_{22}NO_4$).

EXAMPLE 6

PREPARATION OF COMPOUND OF FORMULA II-19 FROM II-16

[0372] A sample of compound of Formula II-16 (250 mg) was added to an acetone solution of sodium iodide (1.5 g in 10 mL) and the resulting mixture stirred for 6 days. The solution was then filtered through a 0.45 micron syringe filter and injected directly on a normal phase silica HPLC column (Phenomenex Luna 10 μm Silica, 25 cm x 21.2 mm) in 0.95 mL aliquots. The HPLC conditions for the separation of compound formula II-19 from unreacted II-16 employed an isocratic HPLC method consisting of 24% ethyl acetate

and 76% hexane, in which the majority of compound II-19 eluted 2.5 minutes before compound II-16. Equivalent fractions from each of 10 injections were pooled to yield 35 mg compound II-19. Compound II-19: UV (Acetonitrile/H_2O) 225 (sh), 255 (sh) nm; ESMS, *m/z* 406.0 (M+H); HRMS (ESI), *m/z* 406.0513 $[M+H]^+$, Δ_{calc}= -0.5 ppm, $C_{15}H_{21}NO_4I$.

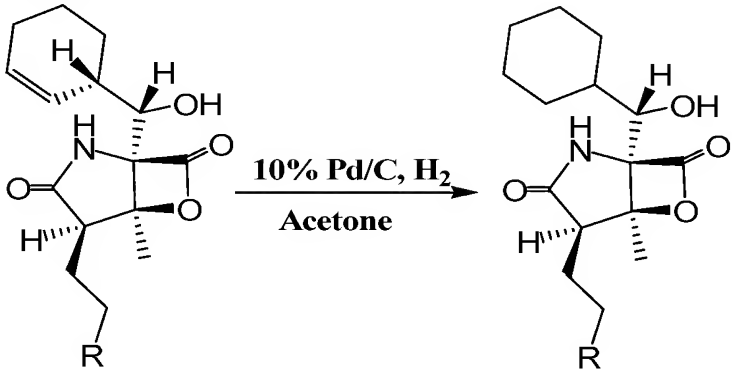


EXAMPLE 7

SYNTHESIS OF THE COMPOUNDS OF FORMULAE II-2, II-3, AND II-4

[0373] Compounds of Formulae II-2, II-3 and II-4 can be synthesized from compounds of Formulae II-16, II-17 and II-18, respectively, by catalytic hydrogenation.

Exemplary Depiction of Synthesis



Example 7A: Catalytic Hydrogenation of Compound of Formula II-16

[0374] Compound of Formula II-16 (10 mg) was dissolved in acetone (5 mL) in a scintillation vial (20 mL) to which was added the 10% (w/w) Pd/C (1-2 mg) and a magnetic

stirrer bar. The reaction mixture was stirred in a hydrogen atmosphere at room temperature for about 15 hours. The reaction mixture was filtered through a 3 cc silica column and washed with acetone. The filtrate was filtered again through 0.2 μm Gelman Acrodisc to remove any traces of catalyst. The solvent was evaporated off from filtrate under reduced pressure to yield the compound of Formula II-2 as a pure white powder: UV (acetonitrile/H_2O): λ_{max} 225 (sh) nm: m/z 316 (M+H), 338 (M+Na).

Example 7B: Catalytic Hydrogenation of Compound of Formula II-17

[0375] Compound of Formula II-17 (5 mg) was dissolved in acetone (3 mL) in a scintillation vial (20 mL) to which was added the 10% (w/w) Pd/C (about 1 mg) and a magnetic stirrer bar. The reaction mixture was stirred in a hydrogen atmosphere at room temperature for about 15 hours. The reaction mixture was filtered through a 0.2 μm Gelman Acrodisc to remove the catalyst. The solvent was evaporated off from filtrate to yield the compound of Formula II-3 as a white powder which was purified by normal phase HPLC using the following conditions:

Column:	Phenomenex Luna 10 μm Silica
Dimensions:	25 cm x 21.2 mm ID
Flow rate:	14.5 mL/min
Detection:	ELSD
Solvent:	5% to 60% EtOAc/Hex for 19 min, 60 to 100% EtOAc in 1 min, then 4 min at 100% EtOAc

[0376] Compound of Formula II-3 eluted at 22.5 min as a pure compound: UV (acetonitrile/H_2O): λ_{max} 225 (sh) nm: m/z 282 (M+H), 304 (M+Na).

Example 7C: Catalytic Hydrogenation of Compound of Formula II-18

[0377] 3.2 mg of compound of Formula II-18 was dissolved in acetone (3 mL) in a scintillation vial (20 mL) to which was added the 10% (w/w) Pd/C (about 1 mg) and a magnetic stirrer bar. The reaction mixture was stirred in hydrogen atmosphere at room temperature for about 15 hours. The reaction mixture was filtered through a 0.2 μm Gelman

Acrodisc to remove the catalyst. The solvent was evaporated off from filtrate to yield the compound of Formula II-4 as a white powder which was further purified by normal phase HPLC using the following conditions:

Column:	Phenomenex Luna 10 μm Silica
Dimensions:	25 cm x 21.2 mm ID
Flow rate:	14.5 mL/min
Detection:	ELSD
Solvent:	5% to 80% EtOAc/Hex for 19 min, 80 to 100% EtOAc in 1 min, then 4 min at 100% EtOAc

[0378] Compound of Formula II-4 eluted at 16.5 min as a pure compound: UV (acetonitrile/H_2O): λ_{max} 225 (sh) nm: m/z 360 (M+H), 382 (M+Na).

[0379] In addition, high resolution mass spectrometry data were obtained for compounds II-2, II-3, and II-4. Compound II-2: HRMS (ESI), *m/z* 316.1305 $[M+H]^+$, Δ_{calc}= -3.5 ppm, $C_{15}H_{23}NO_4Cl$. Compound II-3: HRMS (ESI), *m/z* 282.1706 $[M+H]^+$, Δ_{calc}= 0.3 ppm, $C_{15}H_{24}NO_4$. Compound II-4: HRMS (ESI), *m/z* 360.0798 $[M+H]^+$, Δ_{calc}= -3.4 ppm, $C_{15}H_{23}NO_4Br$.

EXAMPLE 8

SYNTHESIS OF THE COMPOUNDS OF FORMULAE II-5A AND II-5B

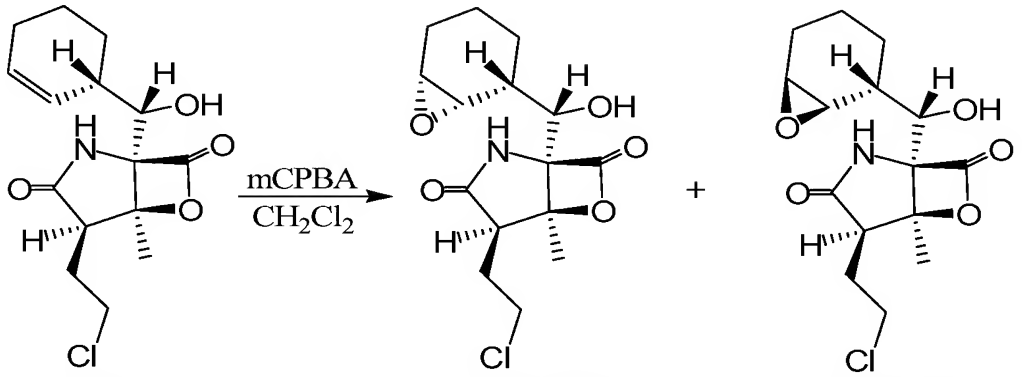
[0380] Compounds of Formula II-5A and Formula II-5B can be synthesized from compound of Formula II-16 by epoxidation with mCPBA.

[0381] Compound of Formula II-16 (101 mg, 0.32 mmole) was dissolved in methylenechloride (30 mL) in a 100 mL of round bottom flask to which was added 79 mg (0.46 mmole) of meta-chloroperbenzoic acid (mCPBA) and a magnetic stir bar. The reaction mixture was stirred at room temperature for about 18 hours. The reaction mixture was poured onto a 20 cc silica flash column and eluted with 120 mL of CH_2Cl_2, 75 mL of 1:1 ethyl acetate/hexane and finally with 40 ml of 100% ethyl acetate. The 1:1 ethyl acetate/hexane fractions yield a mixture of diastereomers of epoxyderivatives, Formula II-5A and II-5B, which were separated by normal phase HPLC using the following conditions:

Column	Phenomenex Luna 10 μm Silica
Dimensions	25 cm x 21.2 mm ID
Flow rate	14.5 mL/min
Detection	ELSD
Solvent	25% to 80% EtOAc/Hex over 19 min, 80 to 100% EtOAc in 1 min, then 5 min at 100% EtOAc

[0382] Compound Formula II-5A (major product) and II-5B (minor product) eluted at 21.5 and 19 min, respectively, as pure compounds. Compound II-5B was further chromatographed on a 3 cc silica flash column to remove traces of chlorobenzoic acid reagent.

Chemical Structures:



II-16 **II-5A (90%)** **II-5B (10%)**

Structural Characterization

[0383] Formula II-5A: UV (Acetonitrile/H_2O) λ_{max} 225 (sh) nm. Low Res. Mass: m/z 330 (M+H), 352 (M+Na); HRMS (ESI), *m/z* 330.1099 $[M+H]^+$, Δ_{calc}= -2.9 ppm, $C_{15}H_{21}NO_5Cl$.

[0384] Formula II-5B: UV (Acetonitrile/H_2O) λ_{max} 225 (sh) nm. Low Res. Mass: m/z 330 (M+H), 352 (M+Na); HRMS (ESI), *m/z* 330.1105 $[M+H]^+$, Δ_{calc}= -0.9 ppm, $C_{15}H_{21}NO_5Cl$.

EXAMPLE 9

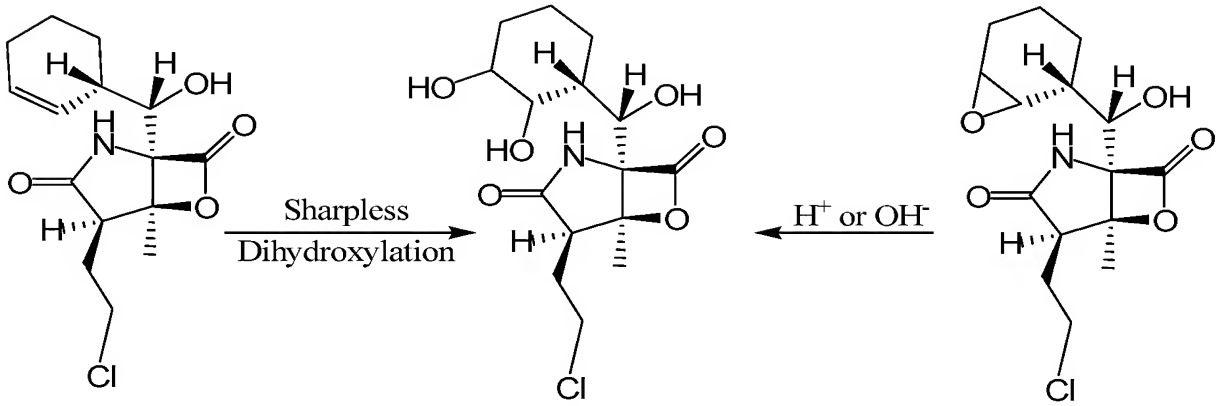
SYNTHESIS OF THE COMPOUNDS OF FORMULAE III-1, III-2, III-3 AND III-4

Synthesis of diol derivatives (Formula III-2)

[0385] Diols can be synthesized by Sharpless dihydroxylation using AD mix-α and β: AD mix-α is a premix of four reagents, $K_2OsO_2(OH)_4$; K_2CO_3; $K_3Fe(CN)_6$; $(DHQ)_2$-PHAL [1,4-bis(9-O-dihydroquinine)phthalazine] and AD mix-β is a premix of $K_2OsO_2(OH)_4$; K_2CO_3; $K_3Fe(CN)_6$; $(DHQD)_2$-PHAL [1,4-bis(9-O-dihydroquinidine)phthalazine] which are commercially available from Aldrich. The diol can also be synthesized by acid or base hydrolysis of epoxy compounds (Formula II-5A and II-5B) which may be different to that of products obtained in Sharpless dihydroxylation in their stereochemistry at carbons bearing hydroxyl groups

Sharpless Dihydroxylation of Compounds II-16, II-17 and II-18

[0386] Any of the compounds of Formulae II-16, II-17 and II-18 can be used as the starting compound. In the example below, compound of Formula II-16 is used. The starting compound is dissolved in t-butanol/water in a round bottom flask to which is added AD mix-α or β and a magnetic stir bar. The reaction is monitored by silica TLC as well as mass spectrometer. The pure diols are obtained by usual workup and purification by flash chromatography or HPLC. The structures are confirmed by NMR spectroscopy and mass spectrometry. In this method both hydroxyl groups are on same side.

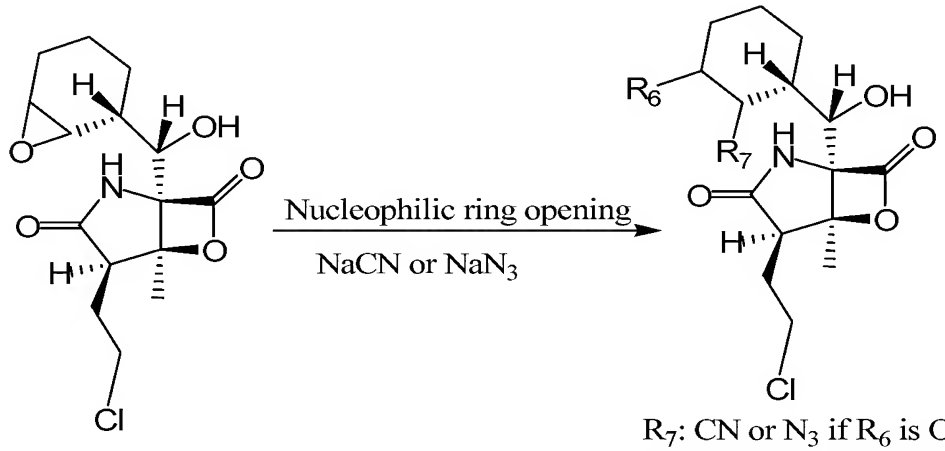


Starting Compound Formula III-2 Formula II-5

Nucleophilic ring opening of epoxy compounds (II-5):

[0387] The epoxy ring is opened with various nucleophiles like NaCN, NaN_3, NaOAc, HBr, HCl, etc. to creat various substituents on the cyclohexane ring, including a hydroxyl substituent.

Examples:

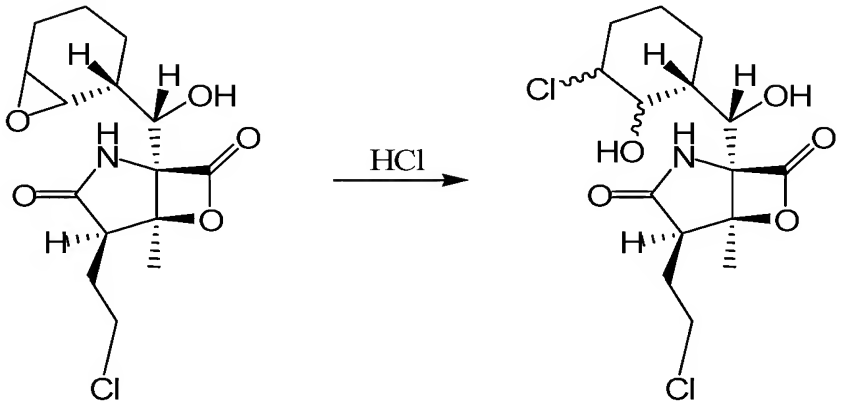


Formula II-5

R_7: CN or N_3 if R_6 is OH
R_6: CN or N_3 if R_7 is OH

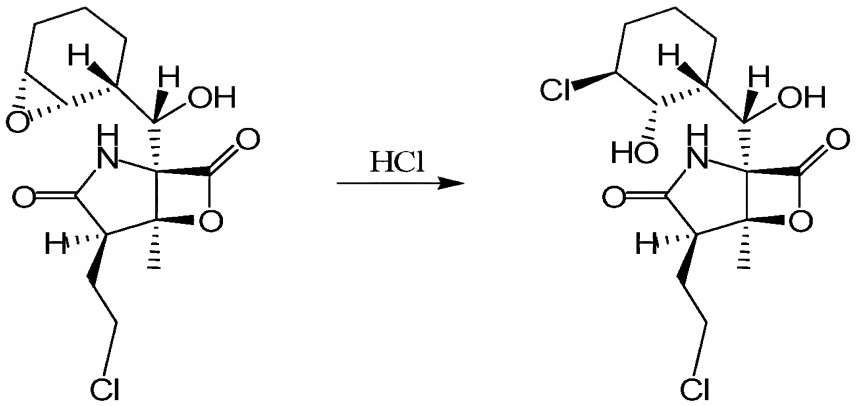
Formula III-1

[0388] The epoxy is opened with HCl to make Formula III-3:



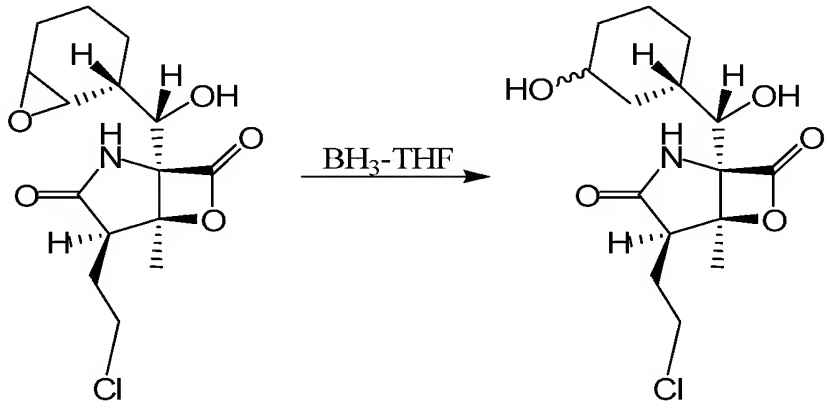
Formula II-5 Formula III-3

[0389] Compound of Formula II-5A (3.3 mg) was dissolved in acetonitrile (0.5 mL) in a 1 dram vial to which was added 5% HCl (500 μL) and a magnetic stir bar. The reaction mixture was stirred at room temperature for about an hour. The reaction was monitored by mass spectrometry. The reaction mixture was directly injected on normal phase HPLC to obtain compound of Formula III-3C as a pure compound without any work up. The HPLC conditions used for the purification were as follows: Phenomenex Luna 10 μm Silica column (25 cm x 21.2 mm ID) with a solvent gradient of 25% to 80% EtOAc/Hex over 19 min, 80 to 100% EtOAc in 1 min, then 5 min at 100% EtOAc at a flow rate of 14.5 mL/min. An ELSD was used to monitor the purification process. Compound of Formula III-3C eluted at about 18 min (2.2 mg). Compound of Formula III-3C: UV (Acetonitrile/H_2O) λ_{max} 225 (sh) nm; ESMS, *m/z* 366 (M+H), 388 (M+Na); HRMS (ESI), *m/z* 366.0875 $[M+H]^+$, Δ_{calc}= 0.0 ppm, $C_{15}H_{22}NO_5Cl_2$. The stereochemistry of the compound of Formula III-3C was determined based on coupling constants observed in the cyclohexane ring in 1:1 C_6D_6/DMSO-d_6.



II-5A **III-3C**

[0390] Reductive ring opening of epoxides (II-5): The compound of Formula is treated with metalhydrides like BH_3-THF complex to make compound of Formula III-4.



Formula II-5 Formula III-4

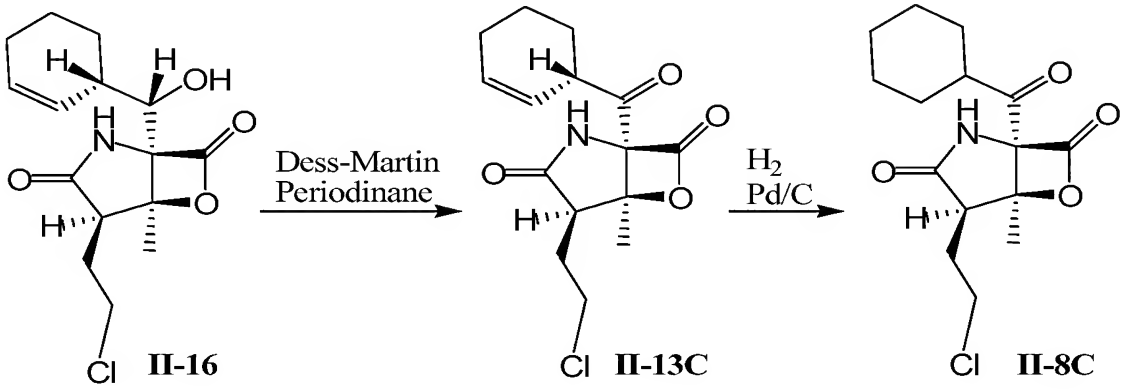
EXAMPLE 10

SYNTHESIS OF THE COMPOUNDS OF FORMULAE II-13C AND II-8C

[0391] Compound of Formula II-16 (30 mg) was dissolved in CH_2Cl_2 (6 mL) in a scintillation vial (20 mL) to which Dess-Martin Periodinane (122 mg) and a magnetic stir bar were added. The reaction mixture was stirred at room temperature for about 2 hours. The progress of the reaction was monitored by TLC (Hex:EtOAc, 6:4) and analytical HPLC. From the reaction mixture, the solvent volume was reduced to one third, absorbed on silica gel, poured on top of a 20 cc silica flash column and eluted in 20 mL fractions using a gradient of Hexane/EtOAc from 10 to 100%. The fraction eluted with 30% EtOAc in Hexane

contained a mixture of rotamers of Formula II-13C in a ratio of 1.5:8.5. The mixture was further purified by normal phase HPLC using the Phenomenex Luna 10 μm Silica column (25 cm x 21.2 mm ID) with a solvent gradient of 25% to 80% EtOAc/Hex over 19 min, 80 to 100% EtOAc over 1 min, holding at 100% EtOAc for 5 min, at a flow rate of 14.5 mL/min. An ELSD was used to monitor the purification process. Compound of Formula II-13C eluted at 13.0 and 13.2 mins as a mixture of rotamers with in a ratio of 1.5:8.5 (7 mg). Formula II-13C: UV (Acetonitrile/H_2O) λ_{max} 226 (sh) & 300 (sh) nm; ESMS, *m/z* 312 $(M+H)^+$, 334 $(M+Na)^+$; HRMS (ESI), *m/z* 312.1017 $[M+H]^+$, Δ_{calc}= 4.5 ppm, $C_{15}H_{19}NO_4Cl$.

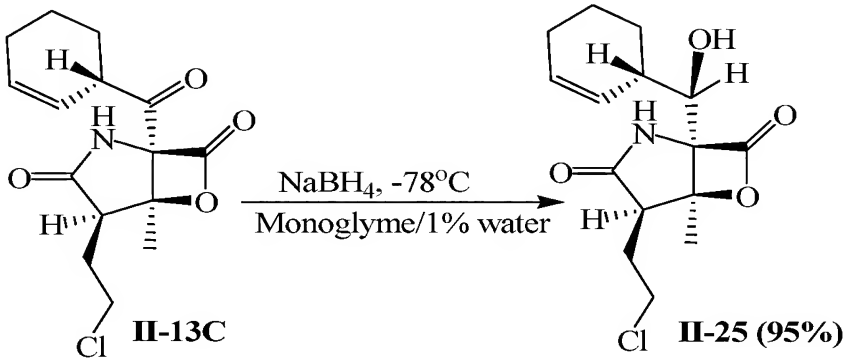
[0392] The rotamer mixture of Formula II-13C (4 mg) was dissolved in acetone (1 mL) in a scintillation vial (20 mL) to which a catalytic amount (0.5 mg) of 10% (w/w) Pd/C and a magnetic stir bar were added. The reaction mixture was stirred in a hydrogen atmosphere at room temperature for about 15 hours. The reaction mixture was filtered through a 0.2 μm Gelman Acrodisc to remove the catalyst. The solvent was evaporated from the filtrate to yield compound of Formula II-8C as a colorless gum which was further purified by normal phase HPLC using a Phenomenex Luna 10 μm Silica column (25 cm x 21.2 mm ID) with a solvent gradient of 25% to 80% EtOAc/Hex over 19 min, 80 to 100% EtOAc over 1 min, holding at 100% EtOAc for 5 min, at a flow rate of 14.5 mL/min. An ELSD was used to monitor the purification process. Compound of Formula II-8C (1 mg) eluted at 13.5 min as a pure compound. Formula II-8C: UV (Acetonitrile/H_2O) λ_{max} 225 (sh) nm; ESMS, *m/z* 314 $(M+H)^+$, 336 $(M+Na)^+$; HRMS (ESI), *m/z* 314.1149 $[M+H]^+$, Δ_{calc}= 3.3 ppm, $C_{15}H_{21}NO_4Cl$.



EXAMPLE 11

SYNTHESIS OF THE COMPOUND OF FORMULA II-25 FROM II-13C

[0393] The rotamer mixture of Formula II-13C (5 mg) was dissolved in dimethoxy ethane (monoglyme; 1.5 mL) in a scintillation vial (20 mL) to which water (15 μL (1% of the final solution concentration)) and a magnetic stir bar were added. The above solution was cooled to −78°C on a dry ice-acetone bath, and a sodium borohydride solution (3.7 mg of $NaBH_4$ in 0.5 mL of monoglyme (created to allow for slow addition)) was added drop-wise. The reaction mixture was stirred at −78°C for about 14 minutes. The reaction mixture was acidified using 2 mL of 4% HCl solution in water and extracted with CH_2Cl_2. The organic layer was evaporated to yield mixture of compound of formulae II-25 and II-16 in a 9.5:0.5 ratio as a white solid, which was further purified by normal phase HPLC using a Phenomenex Luna 10 μm Silica column (25 cm x 21.2 mm ID). The mobile phase was 24% EtOAc/76% Hexane, which was held isocratic for 19 min, followed by a linear gradient of 24% to 100% EtOAc over 1 min, and held at 100% EtOAc for 3 min; the flow rate was 25 mL/min. An ELSD was used to monitor the purification process. Compound of formula II-25 (1.5 mg) eluted at 11.64 min as a pure compound. Compound of Formula II-25: UV (Acetonitrile/H_2O) λ_{max} 225 (sh) nm; ESMS, *m/z* 314 $(M+H)^+$, 336 $(M+Na)^+$; HRMS (ESI), *m/z* 314.1154 $[M+H]^+$, Δ_{calc}= -0.6 ppm, $C_{15}H_{21}NO_4Cl$.



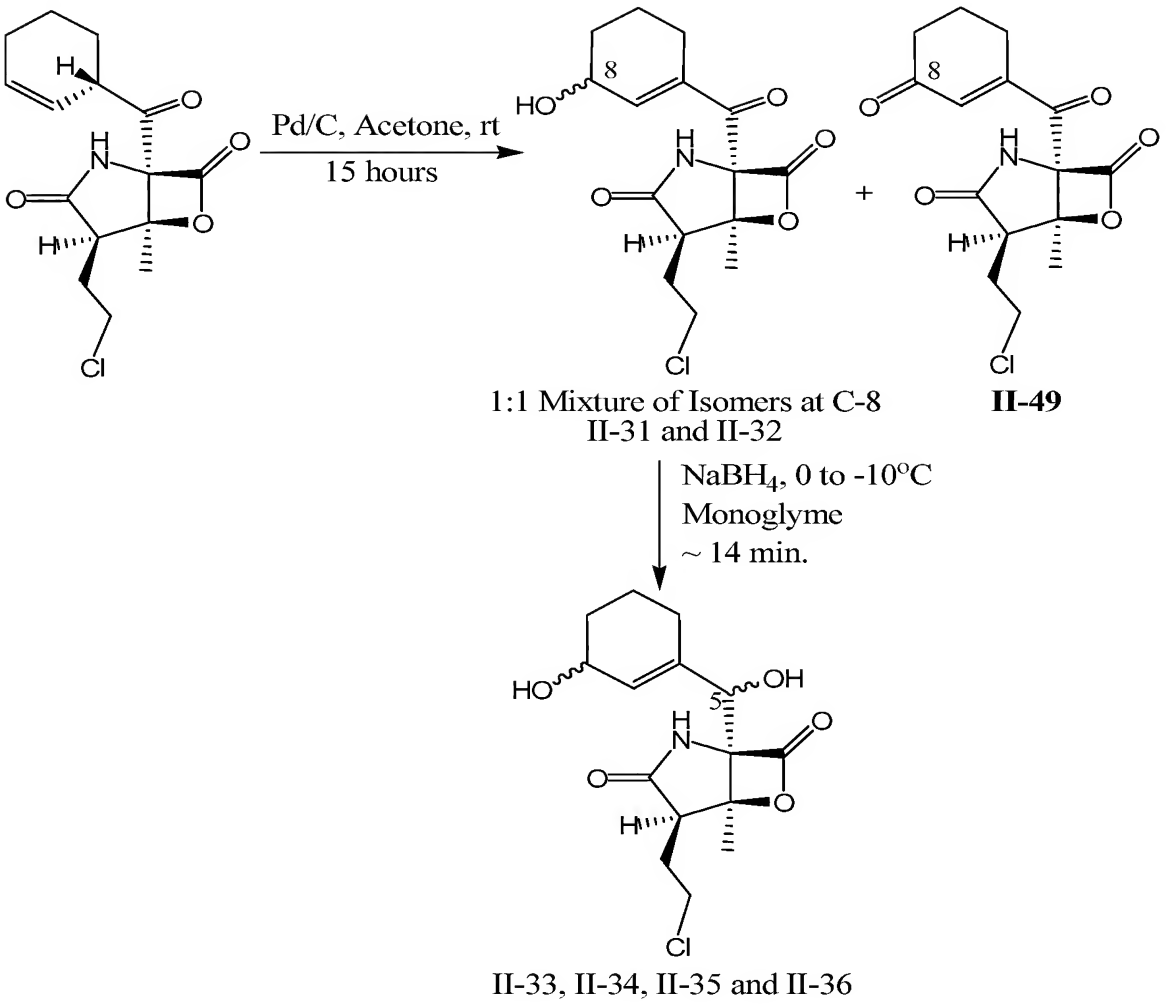
EXAMPLE 12

SYNTHESIS OF THE COMPOUNDS OF FORMULAE II-31, II-32 AND II-49 FROM II-13C; AND COMPOUNDS OF FORMULAE II-33, II-34, II-35 AND II-36 FROM II-31 AND II-32

[0394] A rotamer mixture of the Compound of Formula II-13C (20 mg) was dissolved in acetone (4 mL) in a scintillation vial (20 mL) to which a catalytic amount (3 mg) of 10% (w/w) Pd/C and a magnetic stir bar were added. The reaction mixture was stirred at room temperature for about 15 hours. The reaction mixture was filtered through a 0.2 μm Gelman Acrodisc to remove the catalyst. The solvent was evaporated from the filtrate to yield a mixture of diastereomers of hydroxy derivatives of Formulae II-31 and II-32 (1:1) and a minor compound II-49, which were separated by reversed phase HPLC using Ace 5 μm C18 column (150 mm x 22 mm ID) with a solvent gradient of 90% to 30% H_2O/acetonitrile over 15 min, 70 to 100% acetonitrile over 5 min, holding at 100% acetonitrile for 4 min, at a flow rate of 14.5 mL/min. A diode array detector was used to monitor the purification process. Compound II-31 (2 mg), II-32 (2 mg) and II-49 (0.2 mg) eluted at 10.6, 10.8 and 11.54 min, respectively, as pure compounds. II-31: UV (Acetonitrile/H_2O) λ_{max} 250 (sh) nm; ESMS *m/z* 328.1 $(M+H)^+$ & 350.0 $(M+Na)^+$. II-32: UV (Acetonitrile/H_2O) λ_{max} 250 (sh) nm; ESMS, *m/z* 328.1 $(M+H)^+$ & 350.0 $(M+Na)^+$. II-49: UV (Acetonitrile/H_2O) λ_{max} 250 (sh) and 320 nm; ESMS, *m/z* 326.0 $(M+H)^+$, 343.1 $(M+H_2O)^+$ & 348.0 $(M+Na)^+$.

[0395] In an alternate method, compounds II-31, II-32 and II-49 were separated by normal phase HPLC using Phenomenex Luna 10 μm Silica column (25 cm x 21.2 mm ID) with a solvent gradient of 10% to 100% Hexane/EtOAc over 24 min, holding at 100% EtOAc for 3 min, at a flow rate of 14.5 mL/min. ELSD was used to monitor the purification process.

[0396] The ketone of the compounds of formula II-31 and II-32 can be reduced by using sodium borohydride at 0 to −10°C in monoglyme solvent for about 14 minutes. The reaction mixture can be acidified using 4% HCl solution in water and extracted with CH_2Cl_2. The organic layer can be evaporated to yield the mixtures of compounds of formulae II-33, II-34, II-35 and II-36 which can be separated by chromatographic methods.

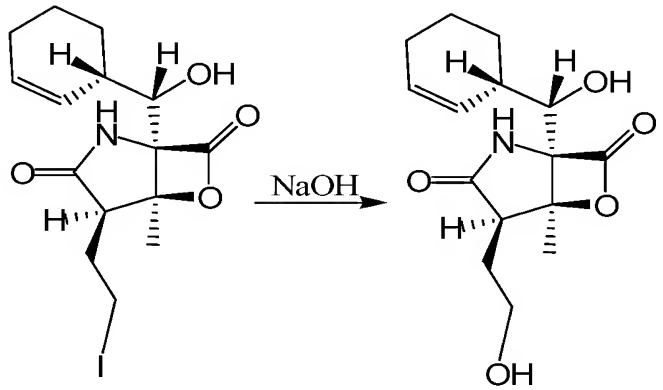


EXAMPLE 13

SYNTHESIS OF THE COMPOUND OF FORMULAE II-21 FROM II-19

[0397] Acetone (7.5 mL) was vigorously mixed with 5 N NaOH (3 mL) and the resulting mixture evaporated to a minimum volume *in vacuo*. A sample of 100 μL of this solution was mixed with compound of Formula II-19 (6.2 mg) in acetone (1 mL) and the resulting biphasic mixture vortexed for 2 minutes. The reaction solution was immediately subjected to preparative C18 HPLC. Conditions for the purification involved a linear gradient if 10% acetonitrile/90% water to 90% acetonitrile/ 10% water over 17 minutes using an Ace 5 μm C18 HPLC column of dimensions 22 mm id by 150 mm length. Compound of

Formula II-21 eluted at 9.1 minutes under these conditions to yield 0.55 mg compound. Compound of Formula II-21: UV (Acetonitrile/H_2O) 225 (sh), ESMS, *m/z* 296.1 (M+H).

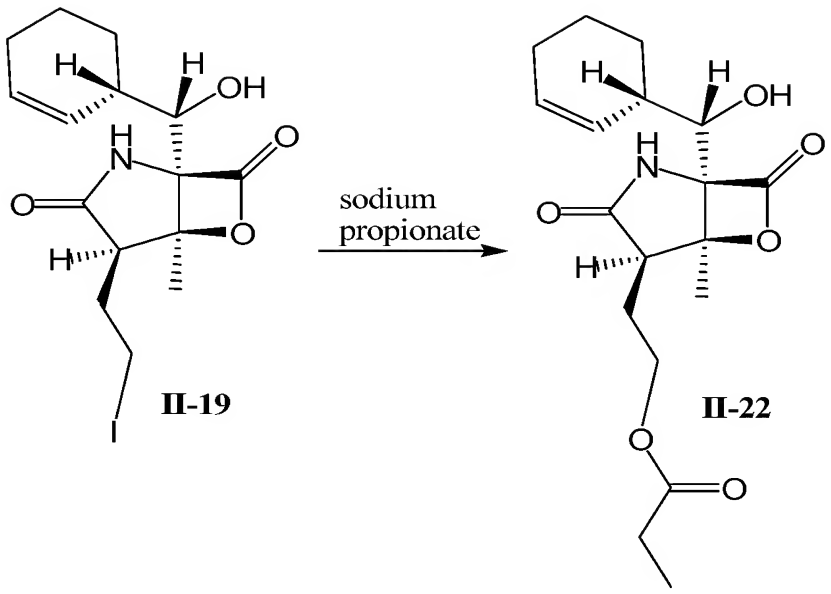


II-19 **II-21**

EXAMPLE 14

SYNTHESIS OF THE COMPOUND OF FORMULAE II-22 FROM II-19

[0398] A sample of 60 mg sodium propionate was added to a solution of compound of Formula II-19 (5.3 mg) in DMSO (1 mL) and the mixture sonicated for 5 minutes, though the sodium propionate did not completely dissolve. After 45 minutes, the solution was filtered through a 0.45 μm syringe filter and purified directly using HPLC. Conditions for the purification involved a linear gradient if 10% acetonitrile/90% water to 90% acetonitrile/10% water over 17 minutes using an Ace 5 μm C18 HPLC column of dimensions 22 mm id by 150 mm length. Under these conditions, compound of Formula II-22 eluted at 12.3 minutes to yield 0.7 mg compound (15% isolated yield). UV (Acetonitrile/H_2O) 225 (sh), ESMS, *m/z* 352.2 (M+H); HRMS (ESI), *m/z* 352.1762 $[M+H]^+$, Δ_{calc}= 0.6 ppm, $C_{18}H_{26}NO_6$.



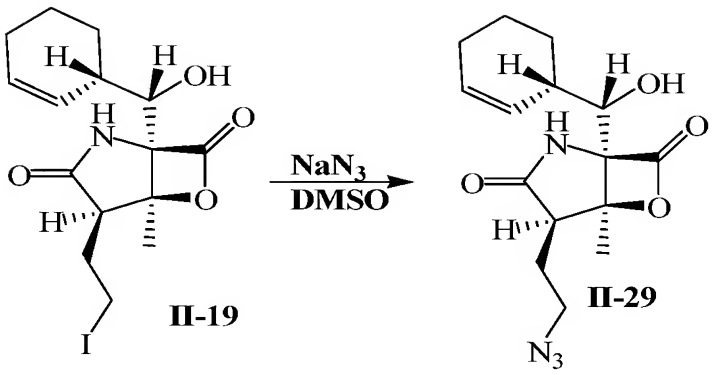
II-19 II-22

EXAMPLE 15

SYNTHESIS OF THE COMPOUND OF FORMULA II-29 FROM II-19

[0399] A sample of NaN_3 (80 mg) was dissolved in DMSO (1 mL) and transferred to a vial containing Compound II-19 (6.2 mg) which was contaminated with approximately 10% Compound II-16. The solution was incubated at room temperature for 1 hr prior to purification on C18 HPLC (ACE 5μm C18-HL, 150 mm X 21 mm ID) using a solvent gradient of 10% acetonitrile/90% H_2O to 90% acetonitrile/10% H_2O over 17 minutes. Using this method, the desired azido derivative II-29 co-eluted with Compound II-16 contaminant at 12.5 minutes (4.2 mg, 85% yield). A 2.4 mg portion of compound II-29 was further purified using additional C18 HPLC chromatography (ACE 5μm C18-HL, 150 mm X 21 mm ID) using an isocratic solvent gradient consisting of 35% acetonitrile/65% H_2O. Under these conditions compound II-29 eluted after 20 minutes, while Compound II-16 eluted after 21.5 minutes. The resulting sample consisted of 1.1 mg Compound II-29 was used for characterization in biological assays.

[0400] Compound II-29: UV (Acetonitrile/H_2O) 225 (sh), ESMS, *m/z* 321.1 (M+H).



II-19 II-29

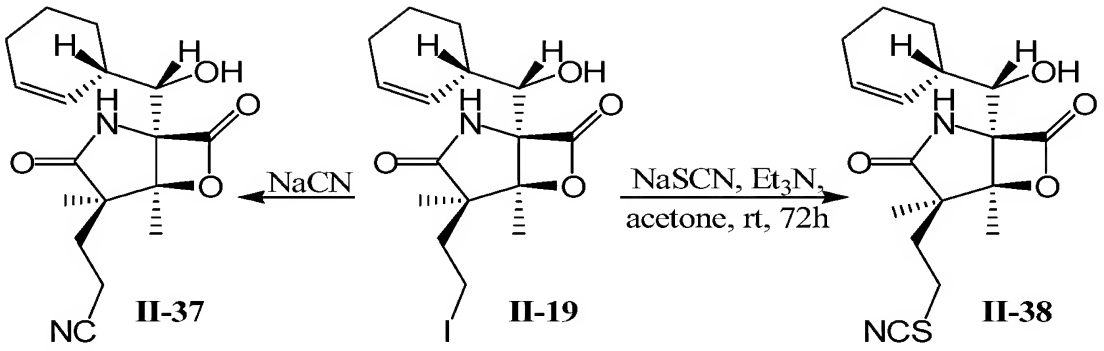
EXAMPLE 16

SYNTHESIS OF THE COMPOUNDS OF FORMULAE II-37 AND II-38 FROM II-19

[0401] The compounds of Formulae II-37 and II-38 can be prepared from the compound of Formula II-19 by cyano-de-halogenation or thiocyanato-de-halogenation, respectively. Compound II-19 can be treated with NaCN or KCN to obtain compound II-37. Alternatively, Compound II-19 can be treated with NaSCN or KSCN to obtain compound II-38.

Synthesis of the compound of Formula II-38 from II-19:

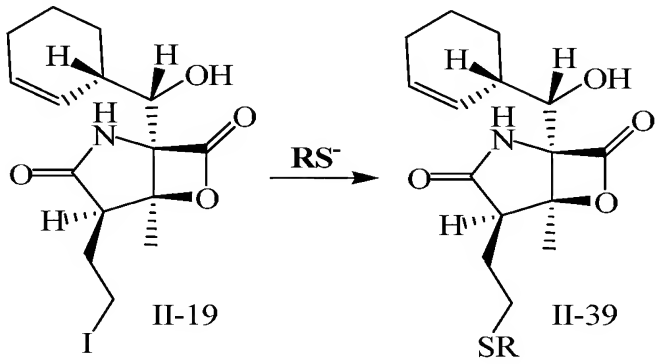
[0402] The compound of formula II-19 (10.6 mg, 0.0262 mmol) was dissolved in 1.5 mL of acetone in a scintillation vial (20 mL) to which sodium thiocyanate (10.0 mg, 0.123 mmol), triethylamine (5 μL, 0.036 mmol) and a magnetic stir bar were added. The reaction mixture was stirred at room temperature for 72 hours. The reaction mixture was concentrated *in vacuo* to yield the compound II-38. Compound II-38 was purified by normal phase HPLC using a Phenomenex Luna 10 μm Silica column (25cm x 21.2 mm ID) with a solvent gradient of 0 to 95% H_2O/acetonitrile over 21 min, at a flow rate of 14.5 mL/min. Diode array detector was used to monitor the purification process. Compound II-38 (3.0 mg, 34% yield) eluted at 18.0 min as a pure compound. II-38: UV Acetonitrile/H_2O λ_{max} 203 (sh) nm; ESMS *m/z* 337.1 $(M+H)^+$ & 359.1 $(M+Na)^+$.



EXAMPLE 17

SYNTHESIS OF THE COMPOUND OF FORMULA II-39 FROM II-19

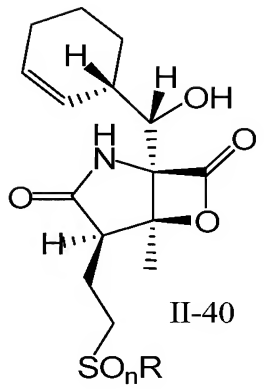
[0403] Thiols and thioethers of the Formula II-39 can be formed by dehalogenation of the compound of Formula II-19. Thiols (R=H) can be formed by treatment of Compound II-19 with NaSH, for example, while thioethers (R=alkyl) can be formed by treatment of Compound II-19 with salts of thiols, or alternatively, by treatment with thiols themselves by running the reaction in benzene in the presence of DBU.



EXAMPLE 18

SYNTHESIS OF THE COMPOUND OF FORMULA II-40 FROM II-39

[0404] Sulfoxides (n=1) and sulfones (n=2) of the Formula II-40 can be formed by oxidation of thioethers of the Formula II-39, for example, with hydrogen peroxide or other oxidizing agents.

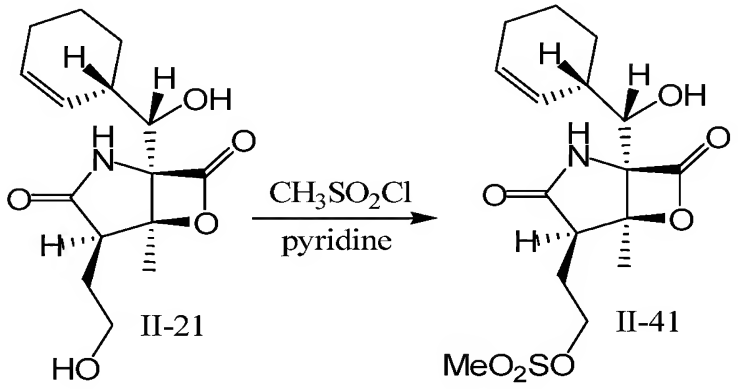


II-40

EXAMPLE 19

SYNTHESIS OF THE COMPOUND OF FORMULA II-41 FROM II-21

[0405] The compound of the Formula II-41 can be prepared by treatment of the compound of Formula II-21 (or a protected derivative of II-21, where the C-5 alcohol or lactam NH are protected, for example) with methyl sulfonyl chloride (mesyl chloride) in pyridine, for example, or by treatment with mesyl chloride in the presence of triethylaminde. Other sulfonate esters can be similarly prepared.

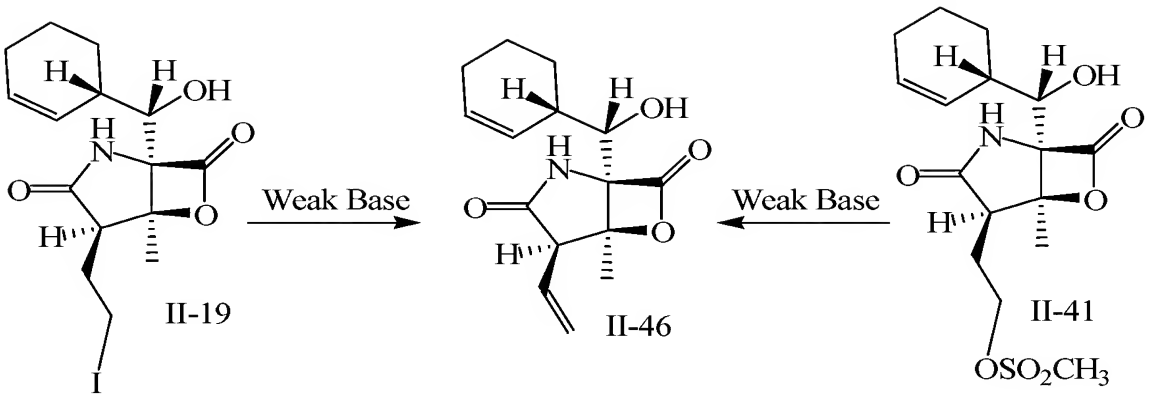


II-21 II-41

EXAMPLE 20

SYNTHESIS OF THE COMPOUND OF FORMULA II-46 FROM II-19 OR II-41

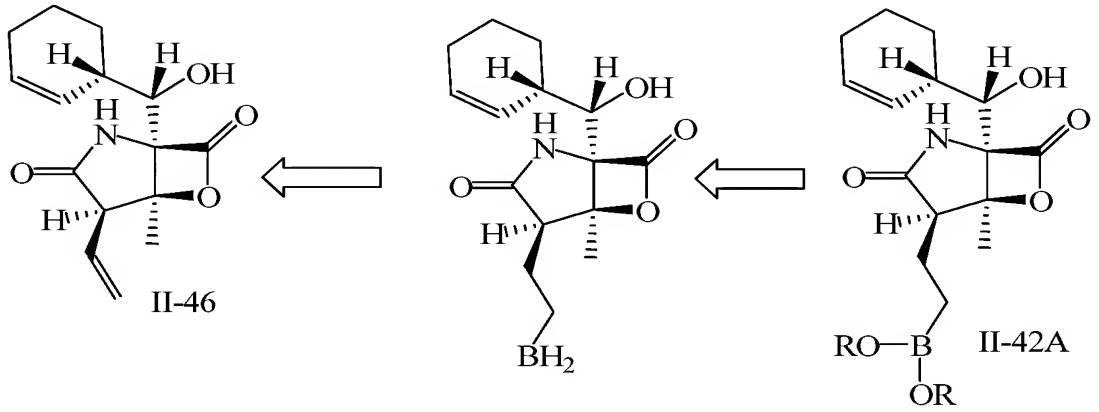
[0406] The alkene of the Formula II-46 can be prepared by dehydroiodination of the compound of Formula II-19, or by hydro-mesyloxy elimination of the compound of Formula II-41, for example, by treatment with base.



EXAMPLE 21

SYNTHESIS OF THE COMPOUND OF FORMULA II-42A

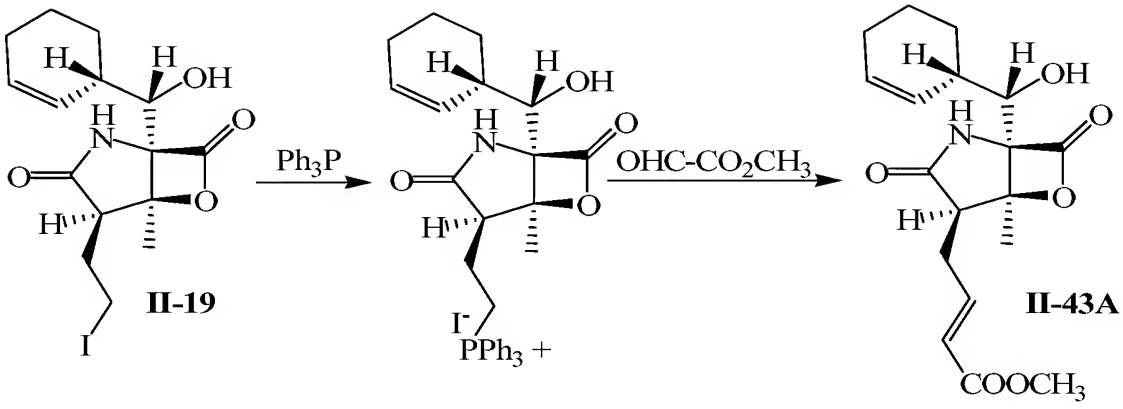
[0407] Synthesis of boronic acids or esters, for example, the compound of the Formula II-42A, can be achieved as outlined in the retrosynthetic scheme below. Hydroboration of the alkene of Formula II-46 gives the corresponding alkyl borane, which can be converted to the corresponding boronic acid or ester, for example, the compound of the Formula II-42A.



EXAMPLE 22

SYNTHESIS OF THE COMPOUND OF FORMULA II-43A

[0408] The compound of the Formula II-43A can be prepared by treatment of the compound of Formula II-19 with triphenyl phosphine to make a phosphorus ylide, which can be treated with various aldehydes, for example, glyoxylic acid methyl ester, to make Formula II-43A.

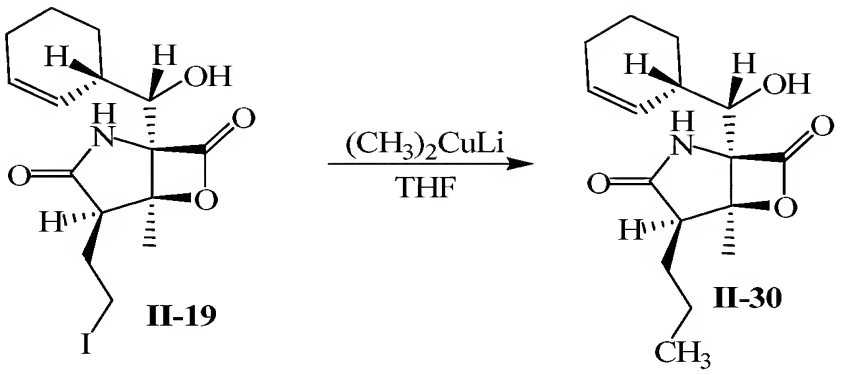


EXAMPLE 23

SYNTHESIS OF THE COMPOUND OF FORMULA II-30 FROM II-19

[0409] A portion of CuI (100 mg) was placed in a 25 mL pear bottom flask and flushed with argon gas for 30 minutes. Argon gas flow was maintained through the flask throughout the course of the reaction. The vessel was cooled to −78°C prior to addition of dry THF (5 mL) followed by the immediate dropwise addition of a solution of methyllithium in dry THF (5.0 mL, 1.6 M) with vigorous stirring. A solution of Compound II-19 in dry THF (12 mg Compound II-19, 1 mL THF) was added slowly to the clear dialkylcuprate solution and the resulting mixture stirred at −78°C for 1 hr. The reaction was quenched by washing the THF solution through a plug of silica gel (1 cm diameter by 2 cm length) along with further washing using a solution of 50% EtOAc / 50% hexanes (50 mL). The combined silica plug washes were dried *in vacuo* and subjected to further C18 HPLC purification in 2 injections (ACE 5 μm C18-HL, 150 mm X 21 mm ID) using an isocratic solvent gradient consisting of 35% acetonitrile/65% H_2O. Compound II-30 eluted under these conditions at 23.5 minutes and yielded 2.4 mg material (27% isolated yield) at 90.8% purity as measured by analytical HPLC. An alternative normal phase purification method can be utilized using Phenomenex Luna 10 μm Silica column (25cm x 21.2 mm ID) with a solvent gradient consisting of 100% hexanes/ethyl acetate to 0% hexanes over 20 minutes. Compound II-30 eluted under these conditions at 16.5 minutes and yielded 3.0 mg material (41% isolated yield) at 97.1% purity as measured by analytical HPLC.

[0410] Compound II-30: UV (Acetonitrile/H_2O) 225 (sh), ESMS, *m/z* 294.1 (M+H); HRMS (ESI), *m/z* 294.1696 $[M+H]^+$, Δ_{calc}= -3.2 ppm, $C_{16}H_{24}NO_4$.



[0411] Compound II-30 can also be obtained by saline fermentation of strain CNB476. In one example, CNB476 was transferred to 500-mL flasks containing 100 mL production medium consisting of the following per liter of deionized water: starch, 10 g; yeast extract, 4 g; Hy-Soy, 4 g; ferric sulfate, 40 mg; potassium bromide, 100 mg; calcium carbonate, 1 g; and synthetic sea salt, 30 g. The production cultures were incubated at 28°C and 250 rpm for 1 day. Approximately 2 g of sterile Amberlite XAD-7 resin was added to the production cultures. The production cultures were further incubated for 5 days. The resin was recovered from the broth and extracted with ethyl acetate. The extract was dried *in vacuo*. The dried extract (8 g) was then processed for the recovery of Compound II-30.

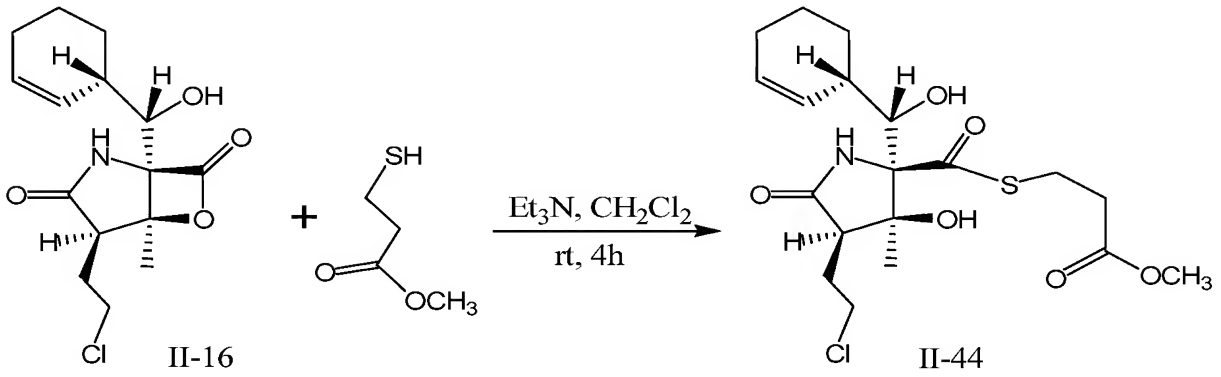
[0412] The crude extract was processed by flash chromatography using a Biotage Flash system. The flash chromatography was developed by the following step gradient: i) Hexanes (1L); ii) 10% EtOAc in hexanes (1L); iii) 20% EtOAc in hexanes, first elution (1L); iv) 20% EtOAc in hexanes, second elution (1L); v) 20% EtOAc in hexanes, third elution (1L); vi) 25% EtOAc in hexanes (1L); vii) 50% EtOAc in hexanes (1L); viii) EtOAc (1L). Fractions containing Compound II-30 was further purified by normal phase HPLC using an isocratic solvent system of 24% EtOAc/hexanes followed by a 100% EtOAc. Compound II-30 eluted 22 minutes into the isocratic portion of the run.

[0413] Fractions enriched in Compound II-30 were further processed by normal phase HPLC using a 27 minute linear gradient from 15% hexanes/85% EtOAc to 100% EtOAc. Compound II-30 eluted after 15 min.

EXAMPLE 24

SYNTHESIS OF THE COMPOUND OF FORMULAE II-44 FROM II-16

[0414] The compound of Formula II-16 (30 mg, 0.096 mmol) was dissolved in CH_2Cl_2 (9 mL) in a scintillation vial (20 mL) to which triethylamine (40 μL, 0.29 mmol), methyl-3-mercapto propionate (thiol, 250 μL) and a magnetic stir bar were added. The reaction mixture was stirred at room temperature for about 4 hours. The solvent was evaporated from the reaction mixture to yield a mixture of compound of Formulae II-44, which was separated by reversed phase HPLC using Ace 5 μm C18 column (150 mm x 22 mm ID) with a solvent gradient of 35% to 90% H_2O/acetonitrile over 17 min, 90 to 100% acetonitrile over1 min, holding at 100% acetonitrile for 1 min, at a flow rate of 14.5 mL/min. Diode array detector was used to monitor the purification process. Compound II-44 (20 mg) eluted at 11.68 min as a pure compound. Compound II-44: UV (Acetonitrile/H_2O) λ_{max} 240 (sh) nm; ESMS *m/z* 434.0 $(M+H)^+$ & 456.0 $(M+Na)^+$.



II-16 II-44

EXAMPLE 25

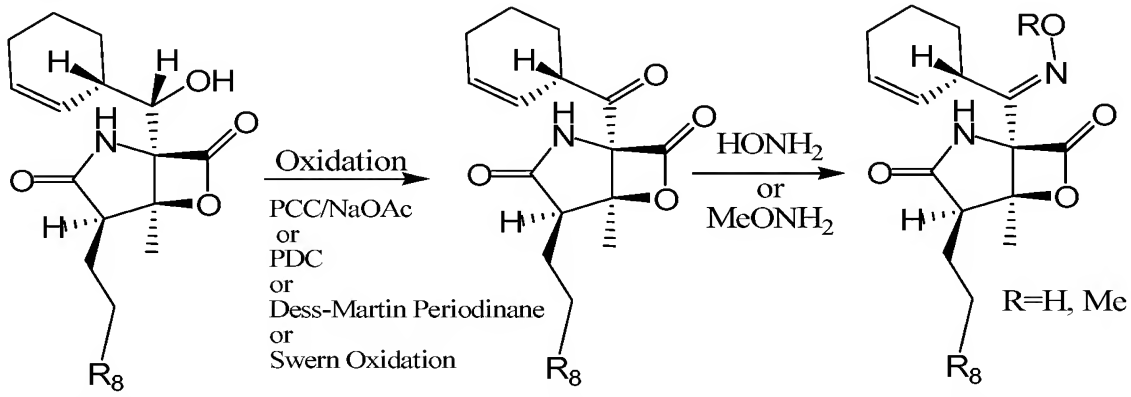
OXIDATION OF SECONDARY HYDROXYL GROUP IN COMPOUNDS OF FORMULAE II-16, II-17 AND II-18

AND REACTION WITH HYDROXY OR METHOXY AMINES

[0415] Any of the compounds of Formulae II-16, II-17 and II-18 can be used as the starting compound. The secondary hydroxyl group in the starting compound is oxidized using either of the following reagents: pyridinium dichromate (PDC), pyridinium chlorochromate (PCC), Dess-Martin periodinane or oxalyl chloride (Swern oxidation) (Ref:

Organic Syntheses, collective volumes I-VIII). Preferably, Dess-Martin periodinane can be used as a reagent for this reaction. (Ref: Fenteany G. *et al.* Science, **1995**, *268*, 726-73). The resulting keto compound is treated with hydroxylamine or methoxy amine to generate oximes.

Examples:



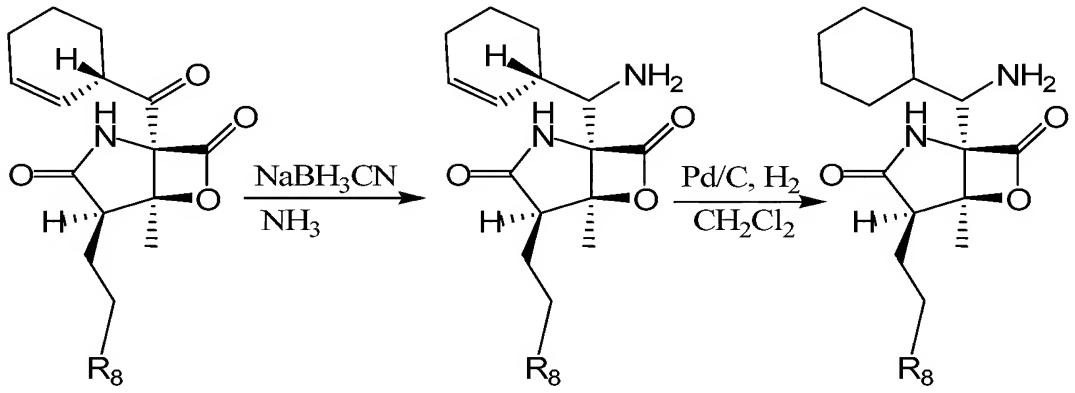
Starting Compound **II-13** **II-9**

EXAMPLE 26

REDUCTIVE AMINATION OF KETO-DERIVATIVE

[0416] The keto derivatives, for example Formula II-8 and II-13, are treated with sodium cyanoborohydride ($NaBH_3CN$) in the presence of various bases to yield amine derivatives of the starting compounds which are subsequently hydrogenated with 10% Pd/C, H_2 to reduce the double bond in the cyclohexene ring.

Example:



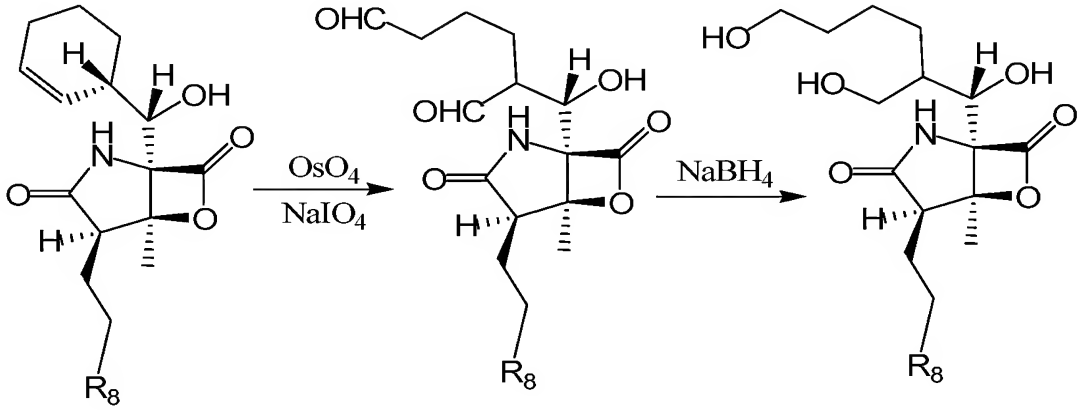
II-13 **II-11** **II-12**

EXAMPLE 27

CYCLOHEXENE RING OPENING

[0417] Any compound of Formulae II-16, II-17 and II-18 can be used as a starting compound. The Starting Compounds can be protected, for example, at the alcohol and/or at the lactam nitrogen positions, and treated with OsO_4 and $NaIO_4$ in THF-H_2O solution to yield dial derivatives which are reduced to the alcohol with $NaBH_4$. The protecting groups can be removed at the appropriate stage of the reaction sequence to produce II-7 or II-6.

Example:

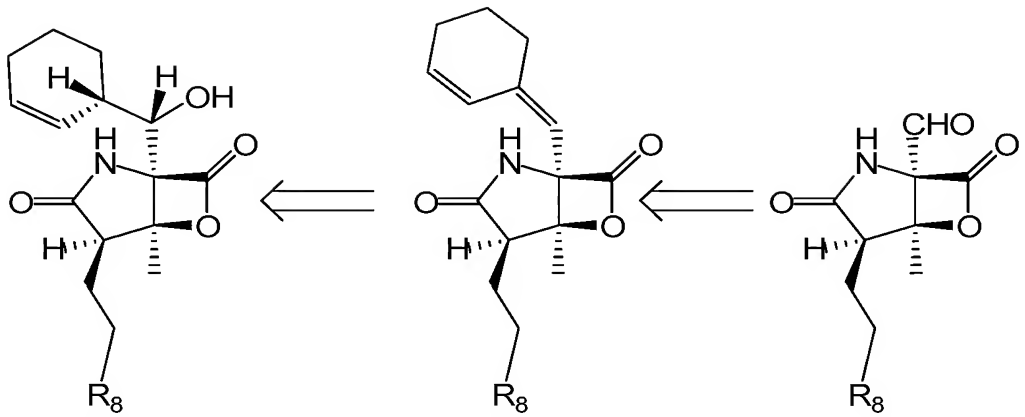


Starting Compound **II-7** **II-6**

EXAMPLE 28

DEHYDRATION OF ALCOHOL FOLLOWED BY ALDEHYDE FORMATION AT LACTONE-LACTAM RING JUNCTION

[0418] A starting compound of any of Formulae II-16, II-17 or II-18 is dehydrated, for example, by treatment with mesylchloride in the presence of base, or, for example, by treatment with Burgess reagent or other dehydrating agents. The resulting dehydrated compound is treated with OsO_4, followed by $NaIO_4$, or alternatively by ozonolysis, to yield an aldehyde group at the lactone-lactam ring junction.

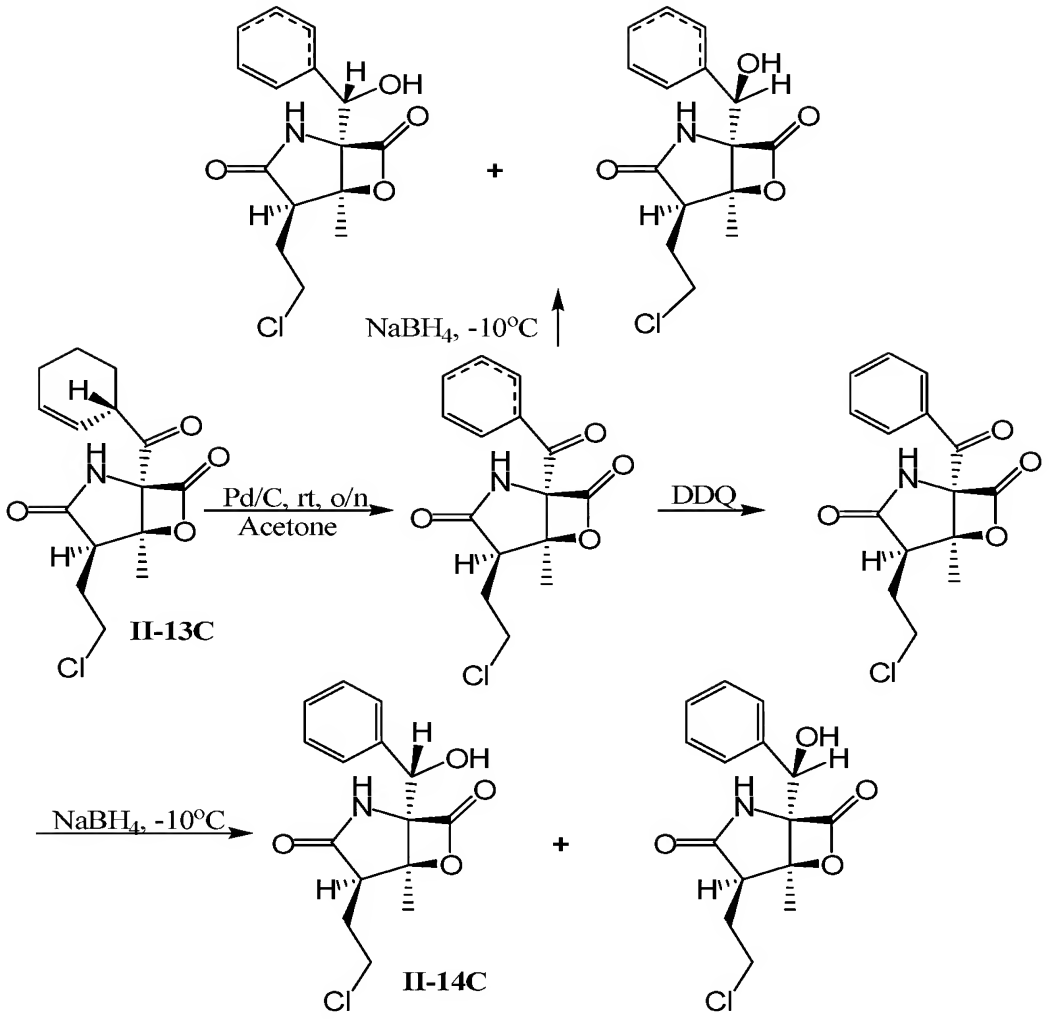


Starting Compound **I-6** **I-1**

EXAMPLE 29

OXIDATION OF THE CYCLOHEXENE RING TO PRODUCE CYCLOHEXADIENES OR A PHENYL RING

[0419] A Starting Compound, such as the ketone of Formula II-13C, is treated with Pd/C to produce a cyclohexadiene derivative. The new double bond can be at any position of the cyclohexene ring. The ketone can be reduced, for example, with sodium borohydride, to obtain the corresponding secondary alcohol(s). Alternatively, the cyclohexadiene derivative can be further treated, for example with DDQ, to aromatize the ring to a phenyl group. Similarly, the ketone can be reduced, for example, with sodium borohydride, to obtain the corresponding secondary alcohol(s).

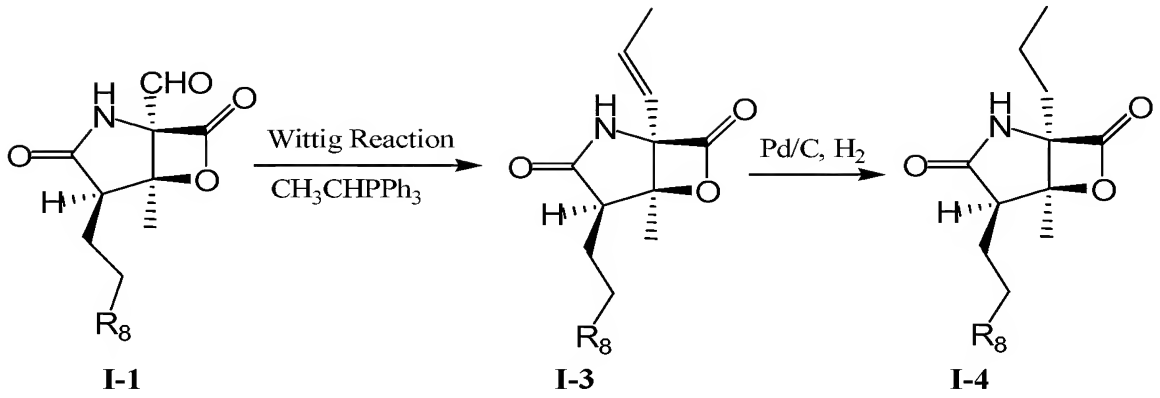


EXAMPLE 30

VARIOUS REACTIONS ON ALDEHYDE DERIVATIVES I-1

[0420] Wittig reactions are performed on the aldehyde group using various phosphorus ylides [*e.g.*, (triphenylphosphoranylidene)ethane] to yield an olefin. The double bond in the side chain is reduced by catalytic hydrogenation.

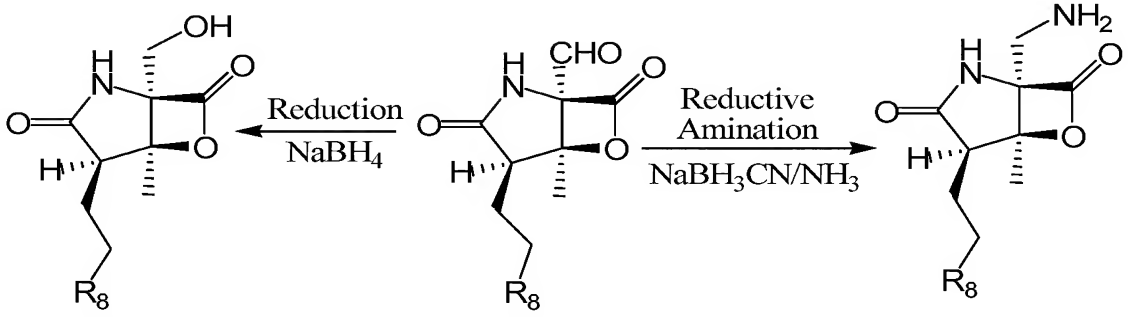
Example:



I-1 **I-3** **I-4**

[0421] Reductive amination is performed on the aldehyde group using various bases (eg. NH_3) and sodium cyanoborohydride to yield amine derivatives. Alternatively, the aldehyde is reduced with $NaBH_4$ to form alcohols in the side chain.

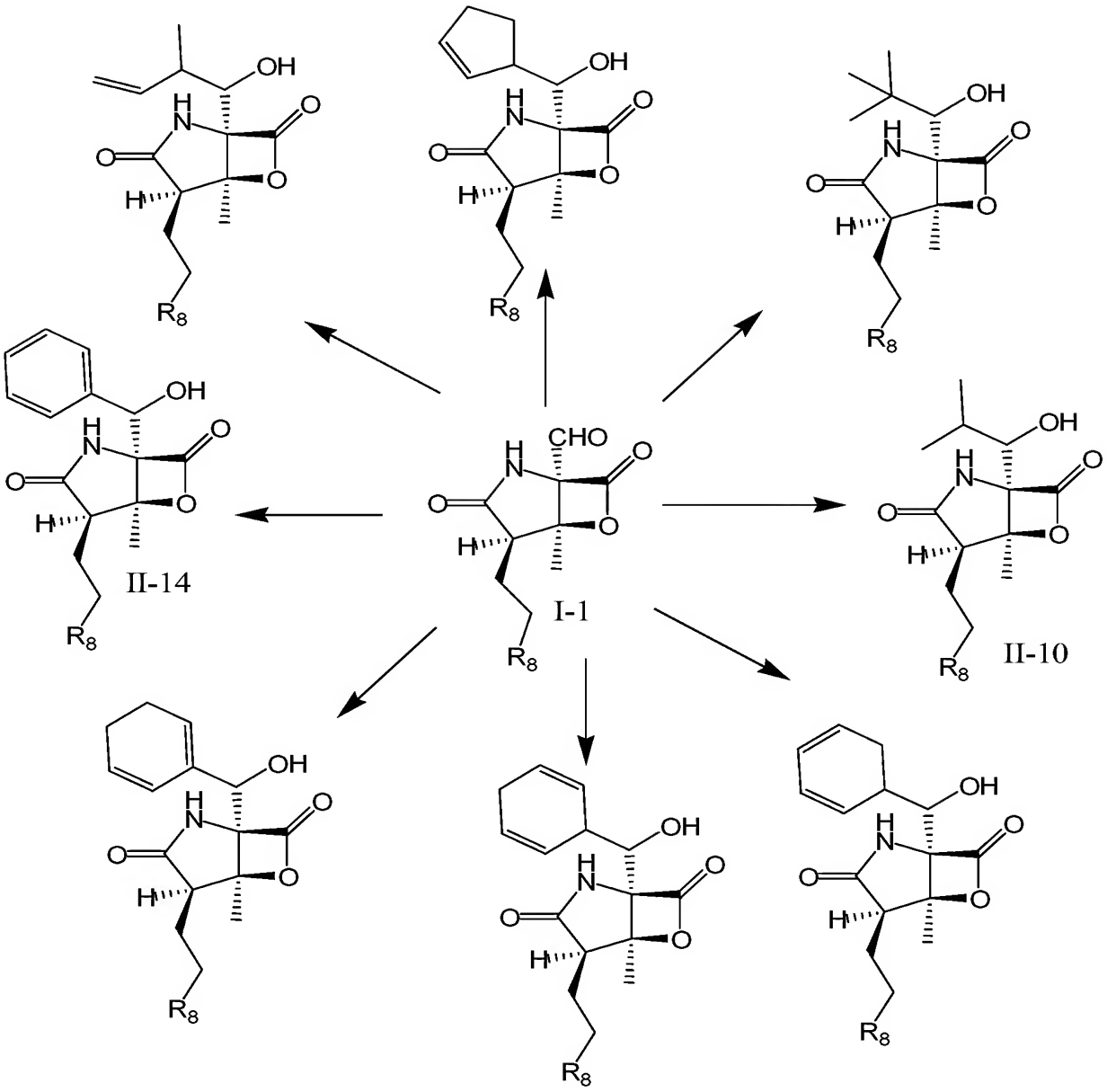
Example:



I-2 **I-1** **I-5**

[0422] Organometallic addition reactions to the aldehyde carbonyl can be performed to yield various substituted secondary alcohols.

Examples:

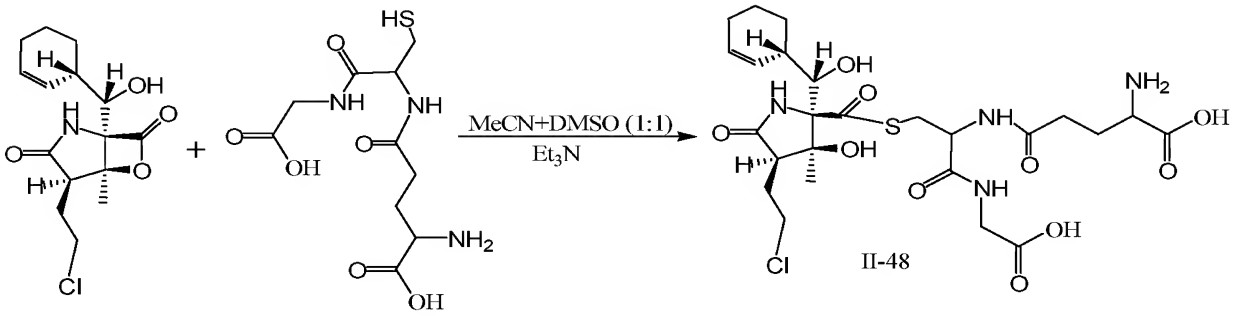


EXAMPLE 31

SYNTHESIS OF THE COMPOUND OF FORMULAE II-48 FROM II-16

[0423] The compound of Formula II-16 (15 mg, 0.048 mmol) was dissolved in 1:1 ratio of acetonitrile/DMSO (8 mL) in a scintillation vial (20 mL) to which triethylamine (40 μL, 0.29 mmol), Glutathione (44.2 mg, 0.144 mmol) and a magnetic stir bar were added. The reaction mixture was stirred at room temperature for about 3 hours. The solvent was

evaporated from the reaction mixture to yield the compound of Formula II-48, which was purified by reversed phase HPLC using Ace 5 μm C18 column (150 mm x 22 mm ID) with a solvent gradient of 10% to 70% H_2O/acetonitrile over 15 min, 70 to 100% acetonitrile over 5 min, holding at 100% acetonitrile for 4 min, at a flow rate of 14.5 mL/min. Diode array detector was used to monitor the purification process. Compound II-48 (10 mg) eluted as a pure compound at 8.255 min. Compound II-48: UV (Acetonitrile/H_2O) λ_{max} 235 (sh) nm; ESMS *m/z* 621.0 $(M+H)^+$.

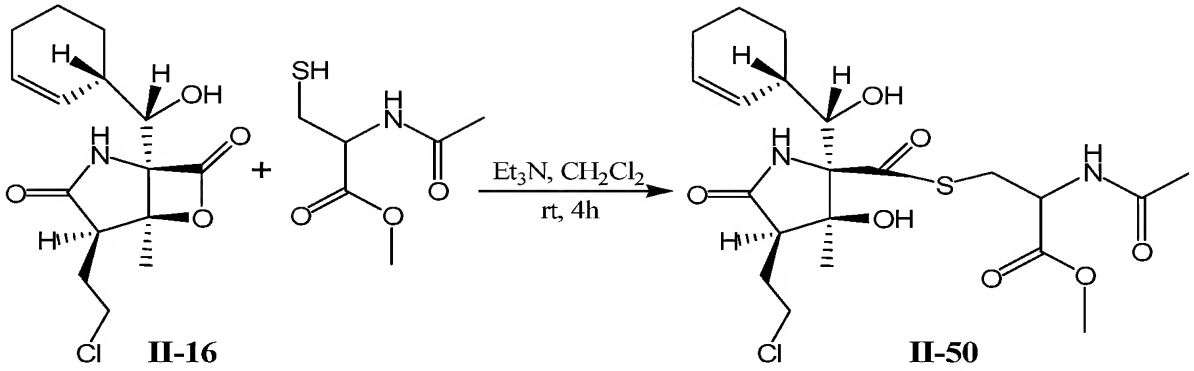


II-16 II-48

EXAMPLE 32

SYNTHESIS OF THE COMPOUND OF FORMULA II-50 FROM II-16

[0424] The compound of Formula II-16 (10 mg, 0.032 mmol) was dissolved in CH_2Cl_2 (9 mL) in scintillation vial (20 mL) to which triethylamine (26.5 μL, 0.192 mmol), N-Acetyl-L-Cysteine methyl ester (17 mg, 0.096 mmol) and a magnetic stir bar were added. The reaction mixture was stirred at room temperature for about 4 hours. The solvent was evaporated from the reaction mixture to yield the mixture of compound of Formulae II-50, which was further purified by normal phase HPLC using Phenomenex Luna 10 μm Silica column (25 cm x 21.2 mm ID) with a solvent gradient of 10% to 100% Hexane/EtOAc over 24 min, holding at 100% EtOAc for 3 min, at a flow rate of 14.5 mL/min. ELSD was used to monitor the purification process. Compound II-50 (2 mg) was eluted at 10.39 min as a pure compounds. Compound II-50: UV (Acetonitrile/H_2O) λ_{max} 230 (sh) nm; ESMS *m/z* 491.1 $(M+H)^+$ & 513.0 $(M+Na)^+$.



II-16 II-50

EXAMPLE 33

FORMULATION TO BE ADMINISTERED ORALLY OR THE LIKE

[0425] A mixture obtained by thoroughly blending 1 g of a compound obtained and purified by the method of the embodiment, 98 g of lactose and 1 g of hydroxypropyl cellulose is formed into granules by any conventional method. The granules are thoroughly dried and sifted to obtain a granule preparation suitable for packaging in bottles or by heat sealing. The resultant granule preparations are orally administered at between approximately 100 mL/day to approximately 1000 mL/day, depending on the symptoms, as deemed appropriate by those of ordinary skill in the art of treating cancerous tumors in humans.

EXAMPLE 34

FORMULATION TO BE ADMINISTERED ORALLY OR THE LIKE

[0426] A mixture obtained by thoroughly blending 1 g of a compound obtained and purified by the method of the embodiment, 98 g of lactose and 1 g of hydroxypropyl cellulose is formed into granules by any conventional method. The granules are thoroughly dried and sifted to obtain a granule preparation suitable for packaging in bottles or by heat sealing. The resultant granule preparations are orally administered at between approximately 100 mL/day to approximately 1000 mL/day, depending on the symptoms, as deemed appropriate by those of ordinary skill in the art of treating cancerous tumors in humans.

EXAMPLE 35

INDUCTION OF APOPTOSIS ALONE AND IN COMBINATION WITH HDAC INHIBITORS

MATERIALS AND METHODS

Cells

[0427] Jurkat, K562, ML-1 and 12.1 (FADD deficient Jurkat) human leukemia cell lines were purchased from American Type Culture Collection (Rockville, MD). Caspase-8 deficient Jurkat cells, 19.2, were obtained from University of Texas, M. D. Anderson Cancer Center, Houston, TX. All cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS (Hyclone, Logan, UT), L-glutamate and penicillin/streptomycin (Sigma, St. Louis, MO). Cells were maintained at 37°C with 5% CO_2. Peripheral blood were obtained from a Philadelphia positive (Ph+) ALL patient. Mononuclear cells were isolated using double density Ficoll-Hypaque gradients composed of Histopaque 1077 and 1119 (Sigma, St. Louis, MO) as previously described (*see* Chandra J, Hackbarth J, Le S, et al. Involvement of reactive oxygen species in adaphostin-induced cytotoxicity in human leukemia cells. Blood. 2003; 1 02:4512-4519).

Reagents

[0428] Salinosporamide A was obtained from Nereus Pharmaceuticals, bortezomib was obtained from the M.D. Anderson Cancer Center pharmacy. HDACi's, (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275) and valproic acid (VPA), were obtained from Calbiochem and Sigma (San Diego, CA and St. Louis, MO). Fluorogenic substrates, suc-LLVY-amc and z-LLE-amc, were from AG Scientific, Inc. (San Diego, CA) and boc-LLR-amc from Bachem (King of Prussia, PA). NAC and staurosporine, were purchased from Sigma (St. Louis, MO). The dyes 6-carboxy-2',7' - dichlorodihydrofluorescein (H2DCF-DA), dihydroethidium (HEt) and tetramethylrhodamine ethyl ester (TMRE) were obtained from Molecular Probes (Eugene, OR). Antibodies were obtained from: Caspase-8, caspase-9, caspase-3, FADD, and Bid (Cell Signaling, Beverly, MA); CH-11 (MBL International, Woburn, MA); p27 (Transduction Laboratories, San Diego, CA); cytochrome c (BD PharMingen, San Diego, CA); and actin (Sigma, St. Louis, MO). The caspase inhibitors zVAD-fmk, IETD-fmk and LEHD-fmk were purchased from

Calbiochem (San Diego, CA). The caspase-3 substrate, DEVD-amc, was obtained from Biomol International, LP (Plymouth Meeting, PA). Annexin V-FITC was purchased from BD Bioscience (Franklin Lakes, NJ).

20S Proteasome Activity Assay

[0429] The chymotrypsin-like, trypsin-like and caspase-like activity of the 20S proteasome of leukemia cells can be determined by measurement of fluorescence generated from the cleavage of the fluorogenic substrates suc-LLVY-amc, boc LRR-amc and z-LLE-amc respectively (*see* Lightcap ES, McCormack TA, Pi en CS, Chau V, Adams J, Elliott PJ. Proteasome inhibition measurements: clinical application. Clin Chem. 2000;46:673-683). Cells were incubated for 1 h in the presence of diluent or 1 /-1M Salinosporamide A, washed with phosphate buffered saline (PBS), and resuspended in 300 /-1L of a solution containing 20 mM Tris, pH 7.5, 0.1 mM EOTA, pH 8.0, 20% glycerol, 0.05% Nonidet-P40, 1 mM 2-13 mercaptoethanol, 1 mM ATP and lysed by freezing and thawing three times on dry ice. After centrifugation, supernatants were combined with substrate buffer (50 mM HEPES, pH 7.5, 5 mM EGTA pH 7) and the specific fluorogenic substrate in a 96-well plate and analyzed on a spectrofluorometer (SpectraMax Gemini EM, Molecular Devices, Sunnyvale, CA) using an excitation of 380 nm and an emission of 460 nm.

Western Blotting

[0430] Cells (5 - 10 x 106) were incubated with indicated concentrations of Salinosporamide A and washed with PBS followed by lysing as previously described (*see* Chandra J, Niemer I, Gilbreath J, et al. Proteasome inhibitors induce apoptosis in glucocorticoid-resistant chronic lymphocytic leukemic lymphocytes. Blood. 1998;92:4220-4229).

[0431] Aliquots of protein lysates (50 /-1g) were run on SOS-polyacrylamide gels. Protein was transferred to nitrocellulose membranes and blocked either for 1 h at room temperature or overnight at 4°C with 5% non-fat dry milk. Membranes were incubated with a 1:1000 dilution of primary antibodies in 5% milk/Tris-buffered saline with 0.05% Tween-20 (TBST), followed by corresponding secondary antibodies (1:1000 dilution with 5% milk/TBST). Bound antibodies were detected using enhanced chemiluminescence (ECL plus

western blotting detection system, Amersham Biosciences UK limited, Little Chalfont Buckinghamshire, England).

Assessment of DNA Fragmentation

[0432] Apoptosis was assessed by propidium iodide (PI) staining followed by fluorescence-activated cell sorting (FACS) analysis as described previously (*see* Chandra J, Hackbarth J, Le S, et al. Involvement of reactive oxygen species in adaphostin-induced cytotoxicity in human leukemia cells. Blood. 2003; 1 02:4512-4519). Following incubation with different doses of Salinosporamide A for 24 h, cells were pelleted by centrifugation and resuspended with PBS containing 50 μg/mL PI, 0.1 % Triton-X-1 00, and 0.1 % sodium citrate. Samples were stored at 4°C for 24 h and vortexed before analysis on the Fl-3 channel of a flow cytometer (FACSCalibur; Becton Dickinson; Franklin lakes, NJ). Data were analyzed using Cell Quest Software (BD Bioscience, Franklin lakes, NJ).

Annexin V staining

[0433] Externalization of phosphatidylserine (PS) was measured by Annexin V-FITC staining according to the manufacturer's protocol. Briefly, 1x106 cells were treated with indicated doses of Salinosporamide A for 6 h, washed twice in cold PBS and resuspended in 1X binding buffer (0.01 M HEPES, pH 7.4; 0.14 M NaCl; 2.5 mM $CaCl_2$) and incubated for 30 min in the dark at room temperature with 5 III Annexin V-FITC and 10 III of 50 μg/mL PI. Samples were analyzed by flow cytometry.

In Vivo studies

[0434] Eight to 12 week old male CB.17/SCID mice were purchased from Taconic Farms (Hudson, NY). SCID mice were injected Lv. (tail vein) with 2 x 107 ML-1 cells in 0.2 mL of PBS. Viability of injected cells was >90% as assessed by trypan blue exclusion. Mice were separated into control and treatment groups (n = 6/group) and treatment with Salinosporamide A (0.15 mg/kg) or vehicle alone (1 % DMSO in PBS) was given intraperitoneally (Lp.) twice a week for 5 weeks. Blood samples were collected on days 17, 21,23, 32 and 35. To avoid tail necrosis, the same mouse was not bled at every time point, accounting for nine bleeds from each group (control versus treated) over the course of 35 days. Complete cell blood counts were performed using a minimum of 50 μL of peripheral blood using a Cell-Dyn (Abbott Diagnostics, Abbott Park, IL) counter. Post hoc power

analysis indicated that with 9 observations per group (control or Salinosporamide A), 26% power was had to see a statistically significant ($p<0.05$) difference in slopes of the regression lines for WBC count (tumor burden) over time. With 36 observations per group 80% power have had to been obtained to see a statistically significant difference between the slopes of the regression lines for WBC count over time.

Transient Transfection

[0435] Full length FADD EGFP-N1 plasmid 30, was transfected into FADD deficient 12.1 cells using Nucleofector Kit V (Amaxa Biosystems, Cologne, Germany) according to the manufacturer's protocol. After 24 h, the brightest 25%GFP positive cells of the total population were sorted by FACS. These GFP positive cells were treated with 200 nM Salinosporamide A for 6 h and analyzed for caspase-3 activity.

Caspase-3 Activity Assays

[0436] Cells were pelleted, resuspended in 150 μL PBS, and lysed by freezing and thawing. Fifty μL was loaded in triplicate on a 96-well plate. To each well, 150 μL of 50 μM DEVD-amc in DEVD Buffer (10% sucrose, 0.001 % IGEPAl, 0.1 % CHAPS, 5 mM HEPES, pH 7.25) was added. Release of fluorescence (amc) was measured using a spectrofluorometer using an excitation of 355 nm and an emission of 460 nm.

Detection of Intracellular Peroxides and Superoxide

[0437] Measurements of intracellular ROS were determined by using cell permeable dyes as previously described 31. Pelleted cells were resuspended in 1 mL of RPMI medium containing either 10 μM CM-H2DCF-DA or 10 μM HEt (measuring intracellular peroxide and superoxide levels, respectively), and incubated at 37°C for 30 min in the dark. Fluorescence intensity was read by flow cytometry on the Fl-1 (DCF) or Fl-3 (HEt) channel.

Measurement of Changes in Mitochondrial Membrane Potential

[0438] Cells were pelleted, washed with PBS, and incubated with 25 nM TMRE in 10 mM HEPES, 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1.8 mM $CaCl_2$, pH 7.4 in a volume of 1 mL for 30 min at 37°C in the dark. Cells were washed in PBS and fluorescence intensity was analyzed on the FL-2 channel of a flow cytometer.

Statistical Analyses

[0439] Values represent the mean S.D. from three separate studies performed in triplicate. Differences in groups were assessed by using paired Student's t-test and were considered statistically significant at $p < 0.05$. For the experiments combining proteasome inhibitors and HDACi, synergism was determined using isobologram analysis based on the Chou and Talalay method with Calcusyn (Biosoft, Ferguson, MO) software program 32. A combination index (CI) value <1.0 indicates synergism: from 0.1-0.3 indicates strong synergism, and from 0.3-0.7 synergism. A CI=1.0 indicates additive effects. In vivo data were analyzed with linear regression to estimate the rate of change (i.e., the slope of the regression line) of the WBC count over time for the mice treated with diluent or Salinosporamide A.

RESULTS

[0440] Salinosporamide A inhibits the 20S proteolytic activities in leukemia cells. To measure Salinosporamide A's effects on the proteolytic activities of the 208 proteasome in leukemia cells, cell lines representative of CML, ALL and AML (K562, Jurkat and ML-1 respectively) were used. Cells were incubated with 1 μM Salinosporamide A for 1 h and the chymotrypsin-like, caspase-like and trypsin-like activities were measured using distinct fluorogenic peptides. Figures 1A and 1B show that 1 μM Salinosporamide A inhibited the chymotrypsin-like and caspase-like activity by greater than 90% compared to diluent in the three leukemia cells lines, whereas the trypsin-like activity was inhibited to a lesser extent (Figure 1C). The accumulation of p27 (Figure 1D) and p21 (data not shown), both known proteasome protein substrates, in cells treated with Salinosporamide A served as functional confirmation of proteasome inhibition.

[0441] The effects of Salinosporamide A and bortezomib on the chymotrypsin-like and caspase-like activities were compared. At the 1 μM dose, Salinosporamide A inhibited both the the chymotrypsin-like and caspase-like activities more effectively than bortezomib ($p = 0.005$ and $p = 0.019$) in Jurkat cells (Figure 1E). To further characterize the ability of Salinosporamide A (NPI-0052) to inhibit proteasome activities in comparison to bortezomib, dose dependent effects of the two inhibitors were assessed (Figure 1E). At the 200 nM dose, Salinosporamide A was more effective than bortezomib at inhibiting the chymotrypsin-like, caspase-like and trypsin-like activities in Jurkat cells.

[0442] Salinosporamide A induces apoptotic cell death in vitro and in vivo. The cytotoxic effects of Salinosporamide A in K562, Jurkat and ML-1 cells were examined next. These cell lines were exposed to 1 nM - 1 μM Salinosporamide A for 24 h. Salinosporamide A induced DNA fragmentation, which peaked at 200 nM, in a dose dependent manner as measured by PI staining in the three leukemia cell lines (Figure 2A). An increase of Annexin V positive and PI negative cells were detected after 6 h of treatment with various doses of Salinosporamide A (19.2% for 10 nM, 28.9% for 50 nM and 28.7% for 200 nM) (Figure 2A inset). Cells were also collected after 1 h exposure with Salinosporamide A and analyzed for inhibition of chymotrypsin-like activity using these same doses. Salinosporamide A doses that induced apoptosis also effectively inhibited the chymotrypsin-like activity in Jurkat cells (Figure 2B). Proteasome inhibition by Salinosporamide A triggered apoptosis in a time dependent fashion, with maximum DNA fragmentation occurring at 24 h (Figure 2C). At a lower dose (10 nM), Salinosporamide A achieved significant proteasome inhibition but did not cause DNA fragmentation. Analysis of phosphatidylserine exposure, an early apoptotic event, at the 10 nM dose, however revealed that 19.2% of cells were Annexin V positive. These findings indicates that higher doses of Salinosporamide A are required to induce apoptosis than those needed to inhibit proteasome activity.

[0443] The ability of Salinosporamide A to exert effects *in vivo* was examined by reconstituting SCID mice with ML-1 cells. Biweekly administration of 0.15 mg/kg Salinosporamide A decreased tumor burden as evidenced by lower total white blood cell counts (WBC; Figure 2D) over the course of five weeks. Linear regression modeling of WBC over time found a difference in the y-intercepts of the regression lines ($p = 0.06$) comparing mice administered Salinosporamide A ($n = 6$ with 9 observations) versus diluent ($n = 6$ with 9 observations). It is worth noting that the slope of the regression line for the Salinosporamide A group was negative (-0.0244) and the slope of the regression line for the control group was positive (0.0317), and that the two lines intersect on the first day of treatment (day 2).

[0444] Since DNA fragmentation is a consequence of caspase-3 activation, caspase-3 activity was examined in response to Salinosporamide A (Figure 3A). Treatment with anti-Fas antibody (CH-11) served as a positive control for caspase-3 activation. As

shown in Figure 3A, 200 nM Salinosporamide A increased caspase-3 activity by 4-fold as compared to control. Inhibition of caspases by a pan-caspase inhibitor, zVAD-fmk, attenuated the Salinosporamide A induced caspase-3 activity. Furthermore, 10 μM IETD-fmk, a caspase-8 inhibitor, abrogated caspase-3 activity by Salinosporamide A ($p < 0.001$), whereas an equimolar dose of caspase-9 inhibitor, LEHD-fmk, did not. The combination of LEHD-fmk and staurosporine was used as a positive control to demonstrate the effectiveness of the caspase-9 inhibitor (Figure 3A). Salinosporamide A induced caspase-3 activity in a time dependent manner, beginning at 4 h and plateauing at 8 h (Figure 3B). Detection of 19 kD and 17 kD cleaved products by Western blot analysis (Figure 3B inset) further confirmed caspase-3 activation by Salinosporamide A.

[0445] Caspase-8 activation by Salinosporamide A. To verify a role for caspase-8 activation as an early event in Salinosporamide A-induced cell death, cleavage of caspase-8 in Jurkat cells was measured (Figure 3C) over a 6 h time period by immunoblotting. Exposure of Jurkat cells to 200 nM Salinosporamide A caused activation of caspase-8 starting at 2 h indicated by the appearance of the 43 kD and 41 kD cleavage fragments.

[0446] The relative contributions of caspase-8 and caspase-9 on Salinosporamide A induced apoptosis were assessed using peptide inhibitors. Jurkat cells were treated with 200 nM Salinosporamide A alone or in combination with caspase-8 and caspase-9 inhibitors for 24 h and DNA fragmentation was assessed. When pretreated with a caspase-8 inhibitor, Jurkat cells were protected against Salinosporamide A induced apoptosis in a statistically significant manner ($p < 0.001$), whereas a caspase-9 inhibitor did not confer protection (Figure 3D).

[0447] Bid is a pro-apoptotic BH-3 domain containing member of the Bcl-2 family and is a substrate for caspase-8 36. Exposure to 1 μM Salinosporamide A for 8 h induced the cleavage of Bid (Figure 3E), generating a 15 kDa fragment, tBid, which can translocate to mitochondria. Thus, while not being bound by any particular theory, Salinosporamide A is likely exerting its cytotoxic effects through a caspase-8-tBid-mitochondria-dependent pathway.

[0448] Mitochondrial injury by Salinosporamide A was evaluated by detecting drops in mitochondrial membrane potential ($\Delta\Psi m$). Reduction in TMRE fluorescence

(indicative of loss of $\Delta\Psi m$) by 56.7% was seen in Jurkat cells treated with 1 μM Salinosporamide A for 6h (Figure 3F). This drop in potential was not abrogated by an antioxidant, NAC. Staurosporine, a positive control, caused a 96.8% reduction in $\Delta\Psi m$. These results support a model in which Salinosporamide A causes caspase-8 activation, leading to Bid cleavage and mitochondrial perturbations.

[0449] Loss of mitochondrial membrane potential generally precedes cytochrome c release from mitochondria which leads to caspase-9 activation in the cytosol 37. Figure 3G shows the presence of cytochrome c in cytosol after 4 h of Salinosporamide A treatment but not in diluent treated cells. Accordingly, levels of pro-caspase-9 were decreased in Salinosporamide A treated cells, indicating activation of the zymogen (Figure 3G).

[0450] Several reports indicate that proteasome inhibition causes increased ROS levels. To determine if ROS levels are heightened by Salinosporamide A, Jurkat cells were treated with 200 nM Salinosporamide A and stained with HEt or DCF. Monitoring ROS over time revealed strong increases in intracellular superoxide and hydrogen peroxide after 14 h of exposure (Figure 3H).

[0451] Requirement for caspase-8 in Salinosporamide A induced apoptosis in leukemia cells. In order to address if caspase-8 is required for Salinosporamide A induced apoptosis, Jurkat cells lacking caspase-8 (I9.2) were utilized and cells lacking the caspase-8 adaptor molecule FADD (I2.1). The absence of caspase-8 and FADD was confirmed by western blotting (Figure 4A). Both of these cell lines showed significant inhibition of chymotrypsin-like proteasome activity after exposure to 1 μM Salinosporamide A (data not shown), indicating that caspase-8 and FADD are not required for Salinosporamide A to inhibit the proteasome.

[0452] Caspase-3 activity was assessed after Salinosporamide A exposure in I2.1 and I9.2 cells. Caspase-8 and FADD deficient cell lines moderately increased caspase-3 activity 3-fold, whereas wild type Jurkat cells exhibited a 6-fold increase as compared to control (Figure 4B). Thus, caspase-8 and FADD mediate Salinosporamide A-induced caspase-3 activity.

[0453] The specific requirement for FADD in Salinosporamide A-induced apoptosis was further tested by transfection of wildtype FADD-GFP into the FADD deficient

I2.1 cells and subsequent treatment with 200 nM Salinosporamide A (Figure 4C). In comparison to parental I2.1 cells, the FADD transfectants displayed higher levels of caspase-3 activity and the difference was statistically significant ($p < 0.05$). As a positive control, 200 ng/mL CH-11 was shown to induce DNA fragmentation in I2.1 FADD-GFP transfectants (53.27%) but not in the I2.1 cell line (4.99%) (Figure 4C inset).

[0454] To place caspase-8 activation in the context of other biochemical apoptotic events, alterations of $\Delta\Psi m$ in parental, caspase-8 deficient and FADD deficient Jurkat cells were examined. Jurkat cells exposed to 200 nM Salinosporamide A displayed a loss of $\Delta\Psi m$ as detected by TMRE staining and indicated by a shift of the histogram (Figure 4D). The caspase-8 and FADD deficient, I9.2 and I2.1, cells did not display a drop in $\Delta\Psi m$ in the presence of Salinosporamide A. These data indicate that caspase-8 and FADD are required for Salinosporamide A to cause mitochondrial perturbations.

[0455] Requirement for Salinosporamide A induced ROS in cytotoxicity and proteasome inhibition. To test if increased levels of ROS by Salinosporamide A contribute to apoptosis, Jurkat cells were treated with 200 nM Salinosporamide A alone or in combination with the antioxidant, NAC. Figure 5A shows that an 8 h exposure to Salinosporamide A caused an increase in caspase-3 activity that was attenuated 3-fold in the presence of NAC. Superoxide levels generated by 100 nM Salinosporamide A were blunted in the presence of NAC (Figure 5A inset). Furthermore, the antioxidant conferred significant protection ($p < 0.001$) against Salinosporamide A induced apoptosis as measured by DNA fragmentation (Figure 5B). Similarly, in mononuclear cells isolated from a Ph+ ALL patient, NAC also protected against cell death by Salinosporamide A (Figure 5C).

[0456] Examination of proteasome activity in Jurkat cells revealed that protection by NAC was not due to interference with Salinosporamide A effects on proteasome function (Figure 5D). In addition, western blot analysis results demonstrate that NAC did not prevent caspase-8 or Bid cleavage in Jurkat cells treated with Salinosporamide A (Figure 5E). Interestingly, neither pan-caspase inhibitors nor specific caspase-8 inhibitors inhibited superoxide production by Salinosporamide A (Figure 5F). It was also observed that NAC does not protect from alterations to mitochondrial membrane potential (Figure 3F). These

results indicate that inhibition of the proteasome and increases in ROS by Salinosporamide A are independent of caspase-8 and Bid activation and mitochondrial perturbations.

[0457] Salinosporamide A interacts with HDACi to induce synergistic apoptosis. It was examined whether the HDACi, MS-275 and VPA, can be combined with Salinosporamide A to enhance apoptosis in leukemia cells. Jurkat cells were treated with increasing doses of MS-275 (1 - 5 μM) or VPA (1 - 5 mM) and low doses of Salinosporamide A (10 nM or 5 nM) for 24 h. The combination of 2.5 μM or 5 μM MS-275 and 10 nM Salinosporamide A significantly increases DNA fragmentation ($p < 0.05$) when compared to cells exposed to a single agent (Figure 6A). Furthermore, cells treated with 10 nM Salinosporamide A and MS-275 had a higher percent increase in the subdiploid population (Figure 6C, $p < 0.05$) and displayed greater synergism (CI = 0.27 for 2.5 11M MS-275 and CI = 0.21 for 5 μM MS-275) compared with cells treated with 10 nM bortezomib and MS-275 (CI = 0.48 for 2.5 μM MS-275 and CI = 0.47 for 5 μM MS-275). To explore the dependence upon caspase-8 of this observed synergy, the comparison of parental Jurkat cells to caspase-8 deficient counterparts was undertaken(I9.2). As predicted by the subdiploid data, caspase-3 activation was potentiated in parental Jurkat cells treated with the combination of Salinosporamide A and MS-275 (Figure 6B; $p < 0.001$). However, caspase-8 deficient cells did not display additive or synergistic effects. Combined treatment of Salinosporamide A and MS-275 resulted in an increase of intracellular superoxide levels (Figure 6B inset). Exposure of Jurkat cells to low doses of VPA (1 or 2.5 mM) and Salinosporamide A (5 nM) also displayed significantly enhanced apoptosis (Figure 6D, $p < 0.05$). As shown in Figure 6D (inset), treatment with 5 nM Salinosporamide A and 2.5 mM VPA resulted in a greater increase in the subdiploid population ($p < 0.05$) as compared with 5 nM bortezomib and VPA. Finally, combination index values indicated synergism between 5 nM Salinosporamide A and 2.5 mM VPA (CI = 0.70), while 5 nM bortezomib and 2.5 mM VPA displayed additive effects (CI = 1.06). These findings demonstrate for the first time that combination of HDACi, (either MS-275 or VPA), with low doses of Salinosporamide A results in synergistic induction of apoptosis and these effects are more potent than those seen when bortezomib is combined with the same agents.

DISCUSSION

[0458] Recent studies have described the effects of Salinosporamide A in myeloma cell lines and animal models, and in primary chronic lymphocytic leukemia (CLL) cells. The current study discloses Salinosporamide A's effects on proteasome activity and the apoptotic machinery in other hematological malignancies, with a focus on ALL and AML model systems. In the current study, it is shown that Salinosporamide A inhibits all three activities associated with the 20S proteasome in leukemia cells (Figure 1) and induces apoptosis in a variety of leukemia cells. Cells representative of ALL, CML, AML, as well as mononuclear cells from a Ph+ ALL patient are sensitive to Salinosporamide A (Figure 2A and 5C). In vivo administration of Salinosporamide A also decreases tumor burden in leukemia bearing mice (Figure 2E). These data demonstrate a spectrum of activity that extends to numerous hematological malignancies.

[0459] Several unique features of Salinosporamide A hint at the potential for efficacy in diseases where bortezomib has been less successful. Firstly, Salinosporamide A inhibits the 20S proteolytic activities in leukemic cells to different degrees, blocking the chymotrypsin-like and caspase-like activities more effectively than the trypsin-like activity (Figure 1A-C). In addition, Salinosporamide A was found to be more potent than bortezomib in inhibiting the rate-limiting activity of the proteasome (Figure 1E). It has been reported that proteasome activities are allosterically regulated and that inhibition of multiple sites of the proteasome is necessary to block significant protein degradation, Salinosporamide A's pattern of inhibition may impact proteasome function.

[0460] The mode of apoptosis induction by Salinosporamide A may also be influenced by this unique differential inhibition of proteasome activities. Caspase-8 inhibition appears to be critical for Salinosporamide A's cytotoxicity whereas caspase-9 inhibitors did not significantly protect against DNA fragmentation or caspase-3 activation (Figure 3A, D). Nevertheless, pro-enzyme caspase-9 disappearance is detected by western blot (Figure 3G) indicating activation of caspase-9 by Salinosporamide A. Since upstream caspases, caspase-8 in this case, can cause activation of other caspases, it is likely that the disappearance of pro-caspase-9 is via this mechanism. These results further suggest that caspase-8 is activating caspase-9 since experiments using caspase-8 inhibitors, or ALL cell lines lacking caspase-8m or FADD showed diminished mitochondrial perturbations, caspase-3 activity, and DNA

fragmentation (Figures 3A, 3D and 4B, 4D). These results place caspase-8 at the apex of the apoptotic cascade triggered by Salinosporamide A (Figure 7). Use of lymphocyte models with total caspase-8 and FADD deficiency, extends and confirms data from multiple myeloma cell lines transfected with dominant- negative caspase-8, caspase-9 or FADD constructs. Furthermore, these studies show Bid cleavage and mitochondrial perturbations by Salinosporamide A in wildtype Jurkat cells, whereas caspase-8 and FADD deficient Jurkat cells do not undergo mitochondrial potential drops (Figure 4D). These results link Salinosporamide A to caspase-8: causing cleavage of Bid, loss of mitochondrial membrane potential, cytochrome c release, caspase-9 activation, caspase-3 activation and DNA fragmentation (Figure 7).

[0461] The antioxidant, NAC, conferred protection against Salinosporamide A induced caspase-3 activity and apoptosis (Figure 5A, 5B). Furthermore, increased peroxide and superoxide levels are detectable after exposure to Salinosporamide A (Figure 3H). Since caspase-8 activation proceeds in the presence of NAC (Figure 5E), and caspase-8 inhibitors do not affect Salinosporamide A's ability to raise intracellular superoxide levels (Figure 5F), these data place ROS alterations in a parallel pathway. Consistent with this model, NAC does not alter proteasome activity (Figure 5D). Several structurally dissimilar proteasome inhibitors are reported to cause elevated levels of intracellular superoxide and intracellular peroxides. The source of this oxidant production is of interest since inhibition of ROS by NAC prevents cytotoxicity in numerous models.

[0462] These results show for the first time that HDACi and low-doses of novel proteasome inhibitor, Salinosporamide A, synergistically induce apoptosis in leukemic cells. Synergistic interactions in leukemia cells exposed to low doses of Salinosporamide A and MS-275 or VPA (Figure 6) were observed. Whereas in cells treated with bortezomib and HOACi, less synergistic and additive effects were observed with MS-275 and VPA, respectively (Figure 6C and 6D inset). The mechanism by which Salinosporamide A synergizes with HDACi still remains to be resolved. However, HDACi have been shown to raise intracellular ROS levels and these results indicate that the combination of Salinosporamide A and MS-275 cause a further increase in superoxide than seen with either agent alone (Figure 6B inset). Thus it is conceivable that this greater oxidative challenge may

contribute to the synergistic effects. These results also implicate caspase-8 in the synergistic apoptosis induced by Salinosporamide A and MS-275 (Figure 6B). This data reveals the potential of administering Salinosporamide A at low doses (nontoxic) with HDACi, such as vorinostat (suberoylanilide hydroxamic acid (SAHA) or Zolinza®), for clinical benefit.

[0463] Taken together, this data suggests that Salinosporamide A is a potent proteasome inhibitor with potential therapeutic value in several hematologic malignancies, such as refractory solid tumors and lymphoma.

EXAMPLE 36

SYNERGISTIC ACTIVITY WITH HISTONE DEACETYLASE INHIBITORS

[0464] For the experiments using Salinosporamide A (NPI-0052) and HDAC inhibitor combinations, synergism was determined using isobologram analysis based on the method by Chou and Talalay (Adv Enzyme Regul 1984, 22: pages 27-55). A combination index (CI) value higher than 1.0 indicates synergism and a CI from 0.1-0.3 indicates strong synergism. A CI of 1.0 indicates additive effects and a CI of >1.0 antagonism.

[0465] Salinosporamide A, and histone deacetylase inhibitors (HDACi) synergistically induce apoptosis in leukemia cell lines in a caspase-8 and oxidant dependent manner. Furthermore, these lethal effects with Salinosporamide A and HDACi were more potent than those obtained when HDACi were combined with the reversible proteasome inhibitor, bortezomib (Velcade). To determine the mechanisms by which Salinosporamide A and HDACi may be synergizing, inhibition of proteasome activity and histone acetylation was examined, the proximal targets of these compounds. Increased acetylation of histone-3 was detected by western blot in Jurkat (T-cell ALL) cells treated with both Salinosporamide A and MS-275 compared to cells exposed to either single agent. Interestingly, these results indicate that the proteasome inhibitor is also eliciting classical chromatin associated epigenetic alterations. In addition, real-time PCR revealed that three structurally different HDACi, MS-275, SAHA (Zolinza/vorinostat) and valproic acid (VPA), decreased expression of the proteasomal β5 subunit, which contains the rate limiting activity of the proteasome: the chymotrypsin-like (CT-L) activity. This reduction of β5 expression affected proteasome function since we detect a decrease in CT-L activity after 12 h with 5 μM MS-275 compared

to cells treated with diluent (P<.01). Further inhibition of CT-L activity was achieved with the combination of 5 μM MS-275 and 0.5 nM Salinosporamide A compared to either treatment alone (P<.005). This effect was reversed by N-acetyl-cysteine (NAC), an antioxidant. Ex vivo combination treatment of mononuclear cells from an AML patient with 5 nM Salinosporamide A and HDACi, MS-275 (1-5 μM) or vorinostat (suberoylanilide hydroxamic acid (SAHA)) (100-500 nM), resulted in an increase of the subdiploid population. Analysis of a range of doses using the Chou and Talalay method determined Salinosporamide A and HDACi treatment displayed greater synergism (CI = 0.241 for 5 μM MS-275 and CI = 0.248 for 500 nM vorinostat (suberoylanilide hydroxamic acid (SAHA))) compared to bortezomib (CI = 0.401 for 5 μM MS-275 and CI = 0.701 for 500 nM vorinostat (suberoylanilide hydroxamic acid (SAHA))). These data, together with the effects of HDACi on β5 subunit expression and proteasome activity, and Salinosporamide A on acetylation reinforces the potential clinical utility of combining these two compounds. Overall, our results suggest that overlapping activities by Salinosporamide A and MS-275 are contributing, along with caspase-8 activation and oxidative stress, to their synergistic cytotoxic effects in leukemia cells.

EXAMPLE 37

EFFECT OF SALINOSPORAMIDE A IN COMBINATION WITH HDAC INHIBITORS ON HEMATOLOGIC MALIGNANCIES

[0466] Salinosporamide A interacts with HDACi (MS-275) to induce synergistic apoptosis. It was examined whether the HDACi, MS-275, can be combined with Salinosporamide A to enhance apoptosis in acute myeloid leukemia (AML) cells. The effect of Salinosporamide A in combination with MS-275 was compared to the effect of Velcade in combination with MS-275. The combination of Salinosporamide A with MS-275 had a greater synergistic effect compared to bortezomib (Velcade) with MS-275 (Figure 8). These findings demonstrate that combination of HDACi, (MS-275), with low doses of Salinosporamide A results in synergistic induction of apoptosis and these effects are more potent than those seen when bortezomib is combined with the same agents(Figure 8).

[0467] Salinosporamide A enhances the activity of vorinostat (SAHA) in Hodgkin's Lymphoma cell lines. Human Reed-Sternberg cell lymphoma (HD-LM2) were incubated with SAHA (1 μM), Salinosporamide A (10 nM) and a combination of SAHA (1 μM) and Salinosporamide A (10 nM). HD-LM2 cell viability was analyzed at 24 hours and 48 hours (Figure 9). The combination of SAHA and Salinosporamide A showed stastical significance at both 24 hours and 48 hours. Similar synergistic results were obtained with Hodgkin's L428 (figure 10A) and KM-H2 (Figure 10B) cell lines.

[0468] Antitumor activity of Salinosporamide A and MS-275 in a human myeloma cell line (RPMI 8226 MM cells) was examined (Figure 11). The effect of combinations of Salinosporamide A (14 nm) with MS-275 (0.5 μM, 2 μM and 3 μM) were found to induce synergism. Additionally, the effect of combination of Salinosporamide A (18 nM) with MS-275 (1 μM) and Salinosporamide A (16 nM) with MS-275 (1.5 μM) were also found to induce synergism. Results are show Table 1 below.

Table 1

MS-275 Dose (μM)	Salinosporamide A Dose (nM)	Mixture Effect	CI
0.5	14	0.26	0.20
1	18	0.30	0.31
1.5	16	0.34	0.38
2	14	0.36	0.45
3	14	0.40	0.54

[0469] Antitumor activity of Salinosporamide A and MS-275 in a human myeloma cell line (OPM-1 MM cells) was examined (Figure 12). The effect of combinations of Salinosporamide A (12 nM, 14 nM and 18 nM) with MS-275 (1.5 μM) were found to induce synergism. Additionally, the effect of combination of Salinosporamide A (18 nm and 20 nm) with MS-275 (2 μM) was also found to induce synergism. The lowest dosage combination, Salinosporamide A (12 nM) and MS-275 (1.5 μM), greatest synergistic effect (CI=0.65). The highest dosage combination, Salinosporamide A (20 nM) and MS-275 (2

μM), had the lowest synergistic effect (CI=0.96). This highest dosage combination had a nearly additive effect. Results are show Table 2 below.

Table 2

MS-275 Dose (μM)	Salinosporamide A Dose (nM)	Mixture Effect	CI
1.5	12	0.53	0.65
1.5	14	0.53	0.73
1.5	18	0.54	0.82
2	18	0.54	0.89
2	20	0.54	0.96

[0470] Antitumor activity of Salinosporamide A and MS-275 in a human myeloma cell line (DHL-6 MM cells) was examined (Figure 13). The effect of combinations of Salinosporamide A (16 nM and 18 nM) with MS-275 (0.5 μM, 1 μM, 1.5 μM and 2 μM) were found to induce synergism. The lowest dosage combination (CI=0.89), Salinosporamide A (16 nM) and MS-275 (0.5 μM), had nearly the same synergistic effect as the highest dosage combination (CI=0.92), Salinosporamide A (18 nM) and MS-275 (2 μM). Results are show Table 3 below.

Table 3

MSX- 275 Dose (uM)	Salinosporamide A Dose (nM)	Mixture Effect	CI
0.5	16	0.62	0.89
1	16	0.66	0.88
	16	0.64	0.93
1.5	18	0.81	0.90
2	18	0.75	0.92

[0471] Antitumor activity of Salinosporamide A and MS-275 in a human myeloma cell line (Dox-6 MM cells) was examined (Figure 14). The effect of combinations of Salinosporamide A (12 nM, 14 nM, and 20 nM) with MS-275 (0.5 μM, 1 μM, 1.5 μM and 2 μM) were found to induce synergism. The lowest dosage of MS-275 (0.5 μM) with the highest dosage of Salinosporamide A (20 nM) had the greatest synergistic effect (CI=0.30). The highest dosage of MS-275 (2 μM) with the lower dosages of Salinosporamide A (12 nM and 14 nM) had lower synergistic effects (CI=0.73 and 0.79, respectiverly). Results are show Table 4 below.

Table 4

MS- 275 Dose (μM)	Salinosporamide A Dose (nM)	Mixture Effect	CI
0.5	20	0.64	0.30
1	20	0.80	0.51
1.5	14	0.92	0.65
2	12	0.97	0.73
2	14	0.95	0.79

[0472] Antitumor activity of Salinosporamide A and MS-275 in a human myeloma cell line (Dox-40 MM cells) was examined (Figure 15). The effect of combinations of Salinosporamide A (14 nM, 16 nM, and 20 nM) with MS-275 (0.5 μM, 1 μM, 1.5 μM and 2 μM) were observed. It was found that Salinosporamide A (14 nM) with MS-275 (0.5 μM) had the largest synergistic effect. At the higher dosage combinations of Salinosporamide A and MS-275 the effects were nearly additive (CI close to 1). Results are show Table 5 below.

Table 5

MS-275 Dose (μM)	Salinosporamide A Dose (nM)	Mixture Effect	CI
0.5	14	0.70	0.76
1	20	0.77	0.98
1.5	16	0.78	0.98
1.5	20	0.81	0.98
2	16	0.81	0.98
2	20	0.84	0.96

[0473] Antitumor activity of Salinosporamide A and MS-275 in a human myeloma cell line (LR-5 MM cells) was examined (Figure 16). The effect of combinations of Salinosporamide A (12 nM, 14 nM, 18 nM and 20 nM) with MS-275 (0.5 μM, 1 μM, and 2 μM) were observed. It was found that Salinosporamide A (12 nM and 14 nM) with MS-275 (0.5 μM, 1 μM and 2 μM) had the largest synergistic effect. The higher dosages of Salinosporamide A (18 nM and 20 nM) with MS-275 (1 μM) had lower synergistic effects. The synergistic effect at the lower dosages of Salinosporamide A (12 nM and 14 nM) was nearly double that of the higher dosages of Salinosporamide A (18 nM and 20 nM). Results are show Table 6 below.

Table 6

MS-275 Dose (μM)	Salinosporamide A Dose (nM)	Mixture Effect	CI
0.5	12	0.44	0.23
1	14	0.45	0.27
1	18	0.41	0.72
1	20	0.42	0.63
2	12	0.46	0.31

[0474] Antitumor activity of Salinosporamide A and MS-275 in a human myeloma cell line (MM.1R cells) was examined (Figure 17). The effect of combinations of Salinosporamide A (3 nM, 5 nM, and 7 nM) with MS-275 (0.25 μM, 0.5 μM, and 1 μM) were observed. The effect of all combinations of Salinosporamide A with MS-275 were found to induce synergism. Although, the combination of Salinosporamide A (3 nM0 with MS-275 (1 μM) was found to be nearly additive (CI=0.91). Results are show Table 7 below.

Table 7

MS-275 Dose (μM)	Salinosporamide A Dose (nM)	Mixture Effect	CI
0.25	5	0.66	0.72
0.25	7	0.72	0.64
0.50	5	0.77	0.70
1	3	0.82	0.91
1	7	0.85	0.83

EXAMPLE 38

EFFECT OF SALINOSPORAMIDE A AND COMBINATIONS WITH HDAC INHIBITORS ON SOLID TUMORS

[0475] Activity of Salinosporamide A and vorinostat on SB-2 (Figure 18A) and WM-266-4 (Figure 18B) melanoma cell lines was observed. The combination of Salinosporamide A (10 nM) with vorinostat (0.5 μM) was compared to Salinosporamide A (10 nM), vorinostat (0.5 μM) and control in the SB-2 and WM-266-4 melanoma cell lines. In the SB-2 melanoma cell line Salinosporamide A (10 nM) and vorinostat (0.5 μM) had nearly the same activity as the control. However, when Salinosporamide A (10 nM) and vorinostat (0.5 μM) were used in combination enhanced activity was observed. In the WM-266-4 melanoma cell line Salinosporamide A (10 nM) and vorinostat (0.5 μM) showed activity greater than the control. Additionally, when Salinosporamide A (10 nM) and vorinostat (0.5 μM) were used in combination synergistic activity was observed. Activity of

Salinosporamide A and vorinostat on MeWo melanoma cell lines was observed (Figure 19). The combination of Salinosporamide A with vorinostat was compared to Salinosporamide A, vorinostat and control in the MeWo melanoma cell lines, the combination of Salinosporamide A (10 nM) with vorinostat (5 μM) increases apoptosis in the MeWo melanoma cell line compared to treatment with the individual agents alone (Figure 19).

[0476] The sensitivity of human lung carcinoma cell lines to Salinosporamide A is shown in Table 8. The IC_{50} values show that Salinosporamide A is active against a variety of human lung tumor types and cell lines. Salinosporamide A was tested at 5 nM to 1 μM and cell growth assessed by MTS assays.

Table 8

Human Lung Tumor Type	Cell Line	IC50 ± SD (nM)
Large Cell Carcinoma	H1341	10 ± 1
Small Cell Carcinoma	H196	20 ± 1
Large Cell Carcinoma	H157	30 ± 2
Squamous Cell Carcinoma	H226	30 ± 3
Adenocarcinoma	H441	50 ± 6
Adenocarcinoma	A549	160 ± 20
Bronchioloalveolar Carcinoma	H322C	280 ± 22
Adenocarcinoma	HCC4006	300 ± 30

[0477] The IC_{50} for Salinosporamide A used alone ranges from about 10 nM (large cell carcinoma H1341 cell line) to about 300 nM (adenocarcinoma HCC4006 cell line). The lung carcinoma cell lines were treated with Salinosporamide A at doses of 5 nM to 500 nM as single agent or in combination with vorinostat (SAHA) (2 μM). At the doses tested, the results indicate that the combination of vorinostat (SAHA) plus Salinosporamide A has additive effects on growth inhibition in the lung cancer cell lines tested (Figures 20 and 21). Additionally, isobologram analysis show the effect of combinations of vorinostat (SAHA) plus Salinosporamide A in various lung carcinoma cell lines: H441 (Figure 22A); A549

(Figure 22B); H322C (Figure 22C); HCC4006 (Figure 22D); H1341 (Figure 22E); H196 (Figure 22F); H157 (Figure 22G); and H226 (Figure 22 H).

[0478] The resistence of human pancreatic carcinoma cell lines to gemcitabine is disclosed (Figure 23). The results indicate that human pancreatic carcinoma cell lines are resistant to treatment with gemcitabine alone. However, when gemcitabine (1 uM or 10 uM) is used at in combination with Salinosporamide A a dosage effect is observed.

[0479] The combination of Salinosporamide A with vorinostat increases apoptosis in human pancreatic carcinoma cell lines (MPanc 96) compared to treatment with the individual agents alone (Figure 24). The results indicate that the combination of Salinosporamide A (20 μM) and vorinostat (10 μM) increased apoptosis more than the combination of Salinosporamide A (50 μM) and vorinostat (10 μM). Increasing the amount of Salinosporamide A with vorinostat kept at a constant dosage (10 μM) did not continue the trend of increasing apoptosis.

EXAMPLE 39

MECHANISMS OF ACTION OF SALINOSPORAMIDE A, HDAC INHIBITORS AND COMBINATIONS OF OF SALINOSPORAMIDE A WITH HDAC INHIBITORS

[0480] The HDAC inhibitor MS-275 can decrease mRNA expression of 20S proteasome β subunits in Jurkat cells (Figure 25). The reletaive expression of 20S proteasome β1, β2, and β5 subunits were all decreased when treated with MS-275 (5 μM) for 24 hours. The HDAC inhibitor vorinostat can decrease mRNA expression of 20S proteasomal β5 subunit in Jurkat cells as measured at 12 hours and 18 hours (Figure 26). The expression of β5 mRNA was analyzed by reqal time PCR were all decreased when treated with MS-275 (5 μM) for 24 hours.

[0481] The HDAC inhibitor MS-275 in combination with Salinosporamide A or bortezomib causes Histone-3 to hyperacetylate (Figure 27). Jurkat T-cells were treated for 6 hours with MS-275 (5 μM), Salinosporamide A (10 nM), bortezomib (10 nM) and combinations of MS-275 (5 μM) with Salinosporamide A (10 nM) or bortezomib (10 nM). It can be seen from the data that the combination treatments cause an increase in the acetylation

of Ac-H3 and H3. Additionally, the data shows that Salinosporamide A (10 nM) causes an increase in the acetylation of H3 relative to control.

[0482] The combination of Salinosporamide A with vorinostat causes hyperacetylation of Histone-3 in jurkat cells (Figure 28). The data shows that N-acetylcysteine (NAC) inhibits the hyperacetylation of Histone-3 in jurkat cells.

[0483] Histone-3 ubiquitination is not affected by NPI-0052 or the combination with MS-275 (Figure 29).

[0484] Synergistic and additive effects of vorinostat pretreatment of human Jurkat ALL cells followed by treatment with bortezomib has been studied (Figure 30). Additionally, synergistic and additive effects of simultaneous treatment with vorinostat and bortezomib has been studied (Figure 31). Synergistic and additive effects of vorinostat pretreatment followed by Salinosporamide A has been studied (Figure 32). Additionally, synergistic and additive effects of simultaneous treatment with vorinostat and Salinosporamide A has been studied (Figure 33). The data shows that treatment of human Jurkat ALL cells with vorinostat and Salinosporamide A produces a greater synergistic effect when the cells are pretreated with vorinostat than when simultaneously treated with vorinostat and Salinosporamide A.

[0485] The effect of MS-275 and Salinosporamide A on superoxide levels in Jurkat T-cells has been studied (Figure 34). Relative to control MS-275 (5 μM) does not increase superoxide levels based on mean fluorescence. Salinosporamide A (10 nM) does increase superoxide levels based on mean fluorescence. The combination of MS-275 (5 μM) and Salinosporamide A (10 nM) provides a synergistic increase in superoxide levels based on mean fluorescence.

[0486] The effect of MS-275 and Salinosporamide A on superoxide levels in Caspase-8 deficient cells has been studied (Figure 35). Relative to control MS-275 (5 μM) does not increase superoxide levels substantially based on mean fluorescence. Salinosporamide A (10 nM) does increase superoxide levels based on mean fluorescence. The combination of MS-275 (5 μM) and Salinosporamide A (10 nM) provides a synergistic increase in superoxide levels based on mean fluorescence.

[0487] The HDAC inhibitor vorinostat in combination with Salinosporamide A or bortezomib causes formation of reactive oxygen species (ROS) (Figure 36). When N-

acetylcysteine (NAC) is included in the combinations the amount of ROS decrease as measured by mean fluorescence. N-acetylcysteine (NAC) decreases formation of ROS when combined with vorinostat and Salinosporamide A but not vorinostat and bortezomib (Figure 37).

[0488] Caspase-8 Activation is required for synergistic apoptosis by Salinosporamide A and vorinostat in ALL Cells (Figure 38). The results show that the combination of vorinostat and Salinosporamide A does not induce apoptosis in cells that are caspase-8 deficient as strongly as cells that are not caspase-8 deficient.

[0489] Regulation of NF-κB Activity *in vitro* by vorinostat and Salinosporamide A combinations (Figure 39). Regulation of NF-κB in human pancreatic carcinoma cells by Salinosporamide A and vorinostat (Figure 40).

EXAMPLE 40

ACTIVITY OF SALINOSPORAMIDE A AND VORINOSTAT COMBINATIONS IN A DRUG RESISTANT ORTHOTOPIC PANCREATIC CARCINOMA TUMOR MODEL

[0490] The combination of Salinosporamide A and vorinostat exhibit enhanced activity in an orthotopic pancreatic tumor model (Figure 41). mPANC-96 is a drug-resistant mesenchymal pancreatic tumor. Luciferase-transduced mPANC-96 cells were used to generate orthotopic tumors in nude mice. The tumor volumes were monitored by bioluminescence imaging after injection of luciferin. Salinosporamide A was administered IP at 0.15 mg/kg weekly. Vorinostat was administered IP 5 days/week at 50 mg/kg. Tumor measurements were made after 3 weeks of therapy. The combination of Salinosporamide A (0.15 mg/kg weekly) and Vorinostat (5 days/week at 50 mg/kg) showed enhanced reduction in tumor volume compared to control.

[0491] The examples described above are set forth solely to assist in the understanding of the embodiments. Thus, those skilled in the art will appreciate that the methods may provide derivatives of compounds.

[0492] One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well

as those inherent therein. The methods and procedures described herein are presently representative of preferred embodiments and are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention.

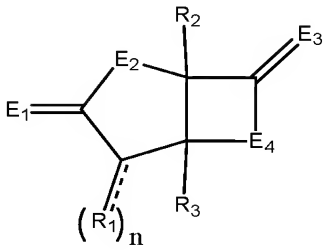
[0493] It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the embodiments disclosed herein without departing from the scope and spirit of the invention.

[0494] All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[0495] The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions indicates the exclusion of equivalents of the features shown and described or portions thereof. It is recognized that various modifications are possible within the scope of the invention. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed can be resorted to by those skilled in the art, and that such modifications and variations are considered to be falling within the scope of the embodiments of the invention.

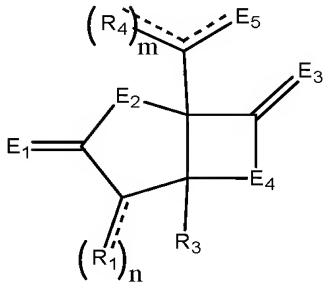
WHAT IS CLAIMED IS:

1. A method of treating cancer comprising administering to an animal a compound having the structure of any one of Formulas I and II, or a pharmaceutically acceptable salt or pro-drug thereof:



Formula I

and



Formula II

in combination with a histone deacetylase inhibitor (HDACi);

wherein:

the dashed lines represent a single or a double bond;

each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

n is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

m is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

$\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide,

sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

$\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

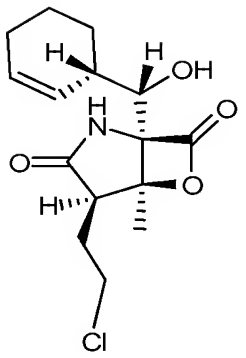
each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

$\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

2. The method of Claim 1, wherein the cancer is selected from the group consisting of breast cancer, sarcoma, leukemia, uretal cancer, bladder cancer, colon cancer, rectal cancer, stomach cancer, lung cancer, lymphoma, liver cancer, kidney cancer, endocrine cancer, skin cancer, melanoma, angioma, brain cancer and central nervous system (CNS) cancer.

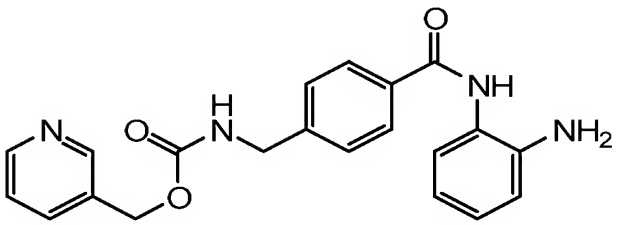
3. The method of any one of the preceding claims, wherein the compound is Salinosporamide A;



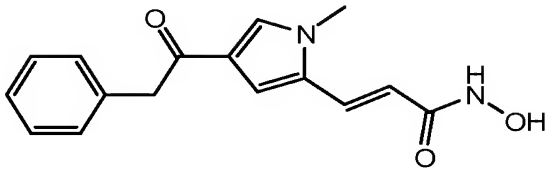
Salinosporamide A

4. The method of any one of the preceding claims, wherein the HDACi and the compound having the structure of any one of Formulas I and II work in a synergistic manner to treat cancer.

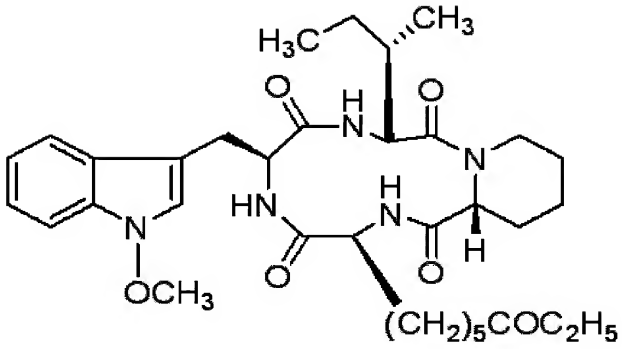
5. The method of any one of the preceding claims, wherein the HDACi is selected from the group consisting of:



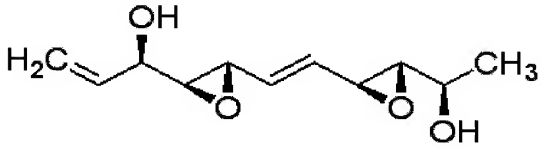
(pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate,



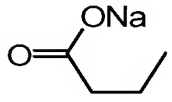
APHA compound 8,



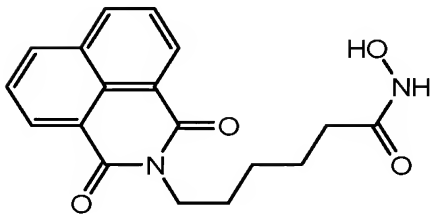
apicidin,



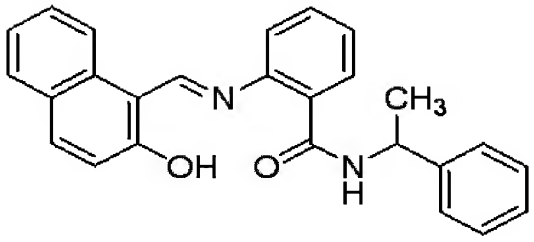
(-)-Depudecin,



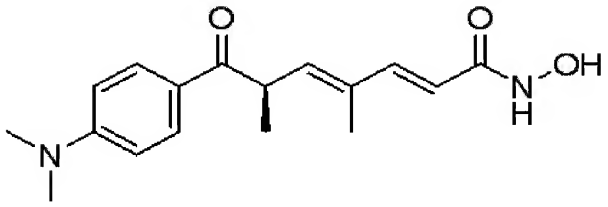
sodium Butyrate,



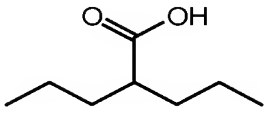
Scriptaid,



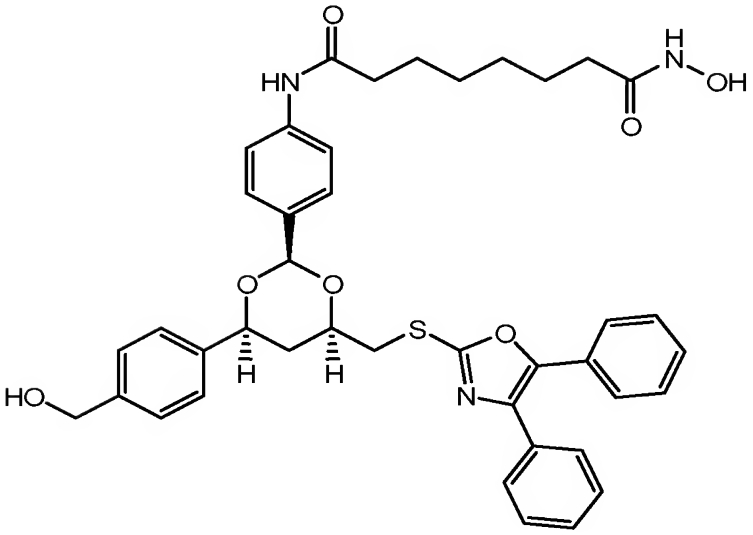
Sirtinol,



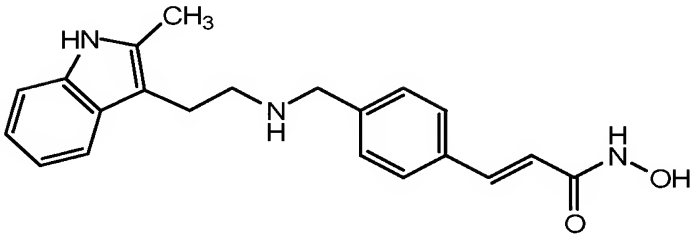
trichostatin A,



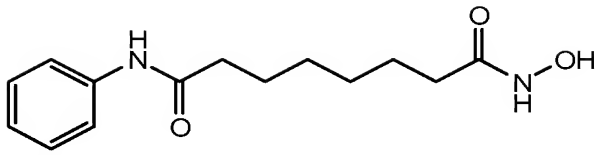
valproic acid,



tubacin,



panobinostat, and



vorinostat (suberoylanilide hydroxamic acid (SAHA)).

6. The method of Claim 5, wherein the HDACi is (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275).

7. The method of Claim 5, wherein the HDACi is valproic acid.

8. The method of Claim 5, wherein the HDACi is vorinostat.

9. The method of any one of the preceding claims, wherein the cancer is leukemia.

10. The method of any one of claims 1-8, wherein the cancer is lymphoma.

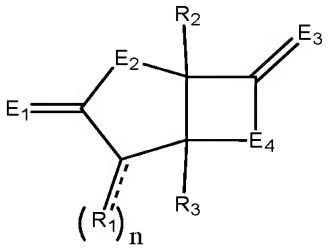
11. The method of any one of the preceding claims, wherein the cancer comprises a tumor.

12. The method of Claim 11, wherein the tumor is a refractory solid tumor.

13. The method of any one of the preceding claims, further comprising co-administering a chemotherapeutic agent.

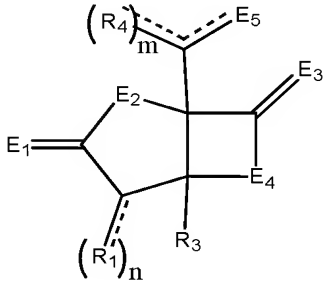
14. The method of any one of the preceding claims, wherein the animal is a human.

15. A pharmaceutical composition comprising a histone deacetylase inhibitor (HDACi) and a compound of any one of Formulas I and II:



Formula I

and



Formula II

wherein:

the dashed lines represent a single or a double bond;

each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

n is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

m is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

$\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

$\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

$\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl,

alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

16. The composition of Claim 15, wherein the compound is Salinosporamide A.

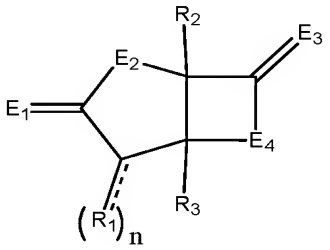
17. The composition of Claim 15 or 16, wherein the HDACi is selected from the group consisting of (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), APHA compound 8, Apicidin, (−)-Depudecin, sodium butyrate, scriptaid, sirtinol, trichostatin A, valproic acid and vorinostat.

18. The composition of Claim 17, wherein the HDACi is (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275).

19. The composition of Claim 17, wherein the HDACi is valproic acid.

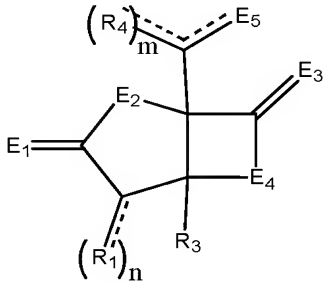
20. The composition of Claim 17, wherein the HDACi is vorinostat.

21. A method of inhibiting the growth of a cancer cell comprising contacting the cell with a HDACi and a compound having the structure of any one of Formulas I and II:



Formula I

and



Formula II

wherein:

the dashed lines represent a single or a double bond;

each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio,

oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

n is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

m is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

$\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

$\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

$\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

22. The method of Claim 21, wherein the compound is Salinosporamide A.

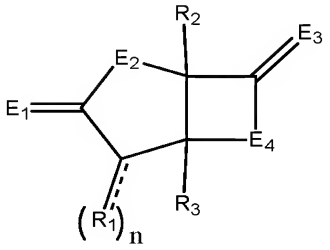
23. The method of Claim 21 or 22, wherein the wherein the HDACi is selected the group consisting of (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), APHA compound 8, apicidin, (−)-Depudecin, sodium butyrate, scriptaid, sirtinol, trichostatin A, valproic acid and vorinostat.

24. The method of Claim 23, wherein the HDACi is (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275).

25. The method of Claim 23, wherein the HDACi is valproic acid.

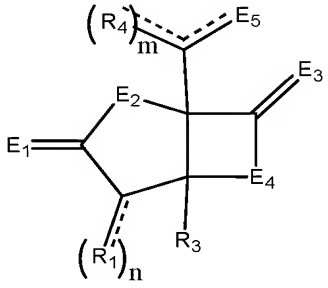
26. The method of Claim 23, wherein the HDACi is vorinostat.

27. A method of inducing apoptosis of a cancer cell comprising contacting the cell with a HDACi and a compound having the structure of any one of Formulas I and II:



Formula I

and



Formula II

wherein:

the dashed lines represent a single or a double bond;

each $\mathbf{R_1}$ is separately a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

n is 1 or 2, where if **n** is 2, then each $\mathbf{R_1}$ can be the same or different;

m is 1 or 2, where if **m** is 2, then each $\mathbf{R_4}$ can be the same or different;

$\mathbf{R_2}$ is a hydrogen, a halogen, a cyano, a nitro, an azido, a hydroxy, or a thiocyano, or selected from the group consisting of optionally substituted: C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl;

$\mathbf{R_3}$ is a halogen or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, aminocarbonyl, aminocarbonyloxy, nitro, azido, phenyl, cycloalkylacyl, hydroxy, alkylthio, arylthio, oxysulfonyl, carboxy, cyano, and halogenated alkyl including polyhalogenated alkyl;

each of $\mathbf{E_1}$, $\mathbf{E_3}$, $\mathbf{E_4}$ and $\mathbf{E_5}$ is an optionally substituted heteroatom;

$\mathbf{E_2}$ is an optionally substituted heteroatom or $-CH_2-$ group; and

each $\mathbf{R_4}$ is separately a halogen, a cyano, a nitro, an azido, or a thiocyano, or selected from the group consisting of optionally substituted C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxycarbonyl, alkoxycarbonylacyl, amino, hydroxy, aminocarbonyl, aminocarbonyloxy, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, boronic acids and esters, and halogenated alkyl including polyhalogenated alkyl.

28. The method of Claim 27, wherein the compound is Salinosporamide A.

29. The method of Claim 27 or 28, wherein the HDACi is selected the group consisting of (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275), APHA compound 8, Apicidin, (−)-Depudecin, sodium butyrate, scriptaid, sirtinol, trichostatin A, valproic acid and vorinostat.

30. The method of Claim 29, wherein the HDACi is (pyridin-3-yl)methyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275).

31. The method of Claim 29, wherein the HDACi is valproic acid.

32. The method of Claim 29, wherein the HDACi is vorinostat.

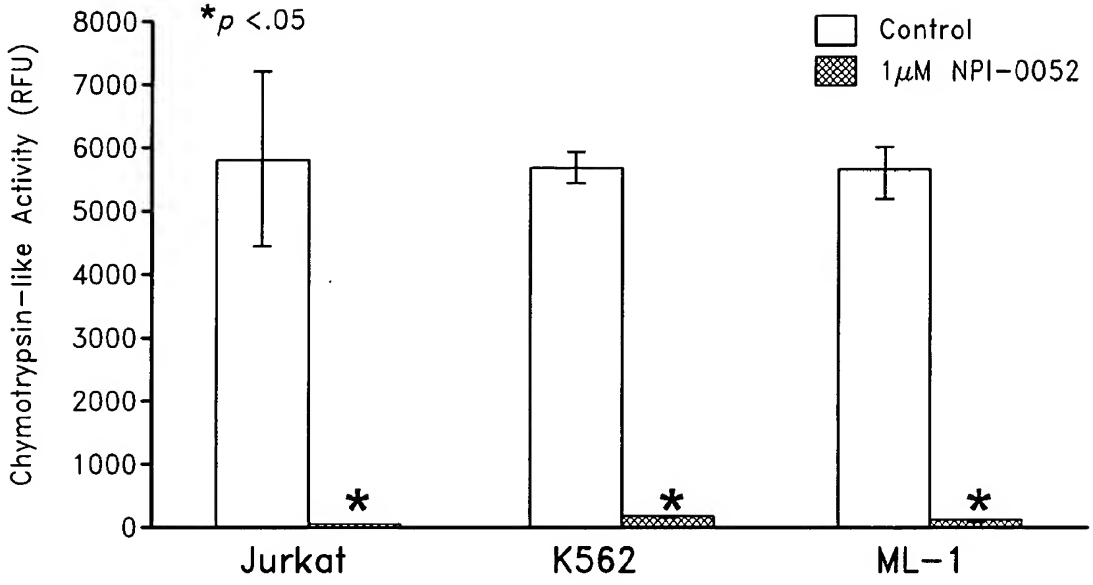

*p <.05
Control
1μM NPI-0052
Chymotrypsin-like Activity (RFU)
8000
7000
6000
5000
4000
3000
2000
1000
0
Jurkat
K562
ML-1

FIG. 1A

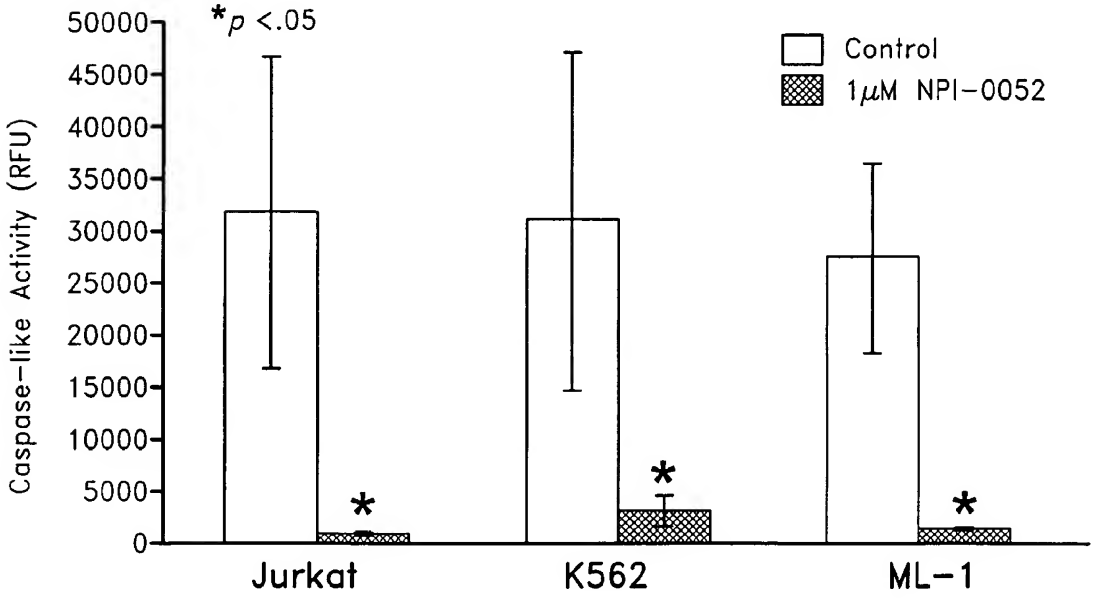

*p <.05
Control
1μM NPI-0052
Caspase-like Activity (RFU)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Jurkat
K562
ML-1

FIG. 1B

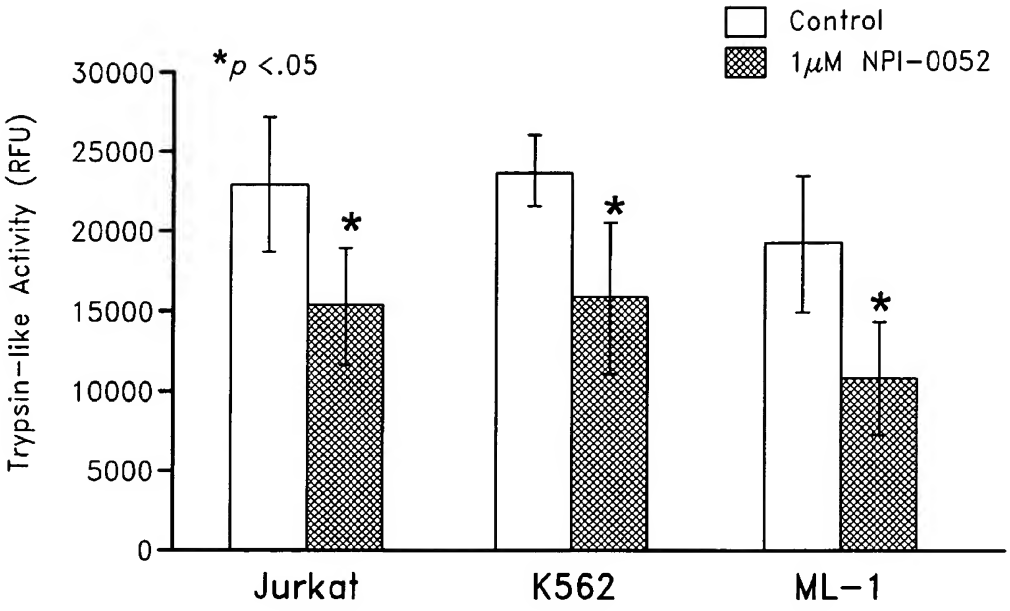

Control
1μM NPI-0052
*p <.05
Trypsin-like Activity (RFU)
30000
25000
20000
15000
10000
5000
0
*
*
*
Jurkat
K562
ML-1

FIG. 1C

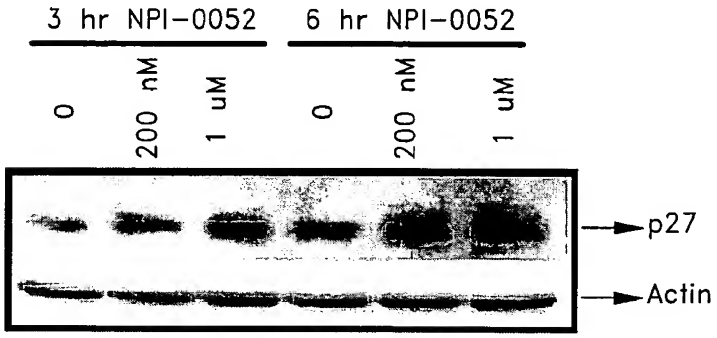

3 hr NPI-0052
6 hr NPI-0052
0
200 nM
1 uM
0
200 nM
1 uM
p27
Actin

FIG. 1D

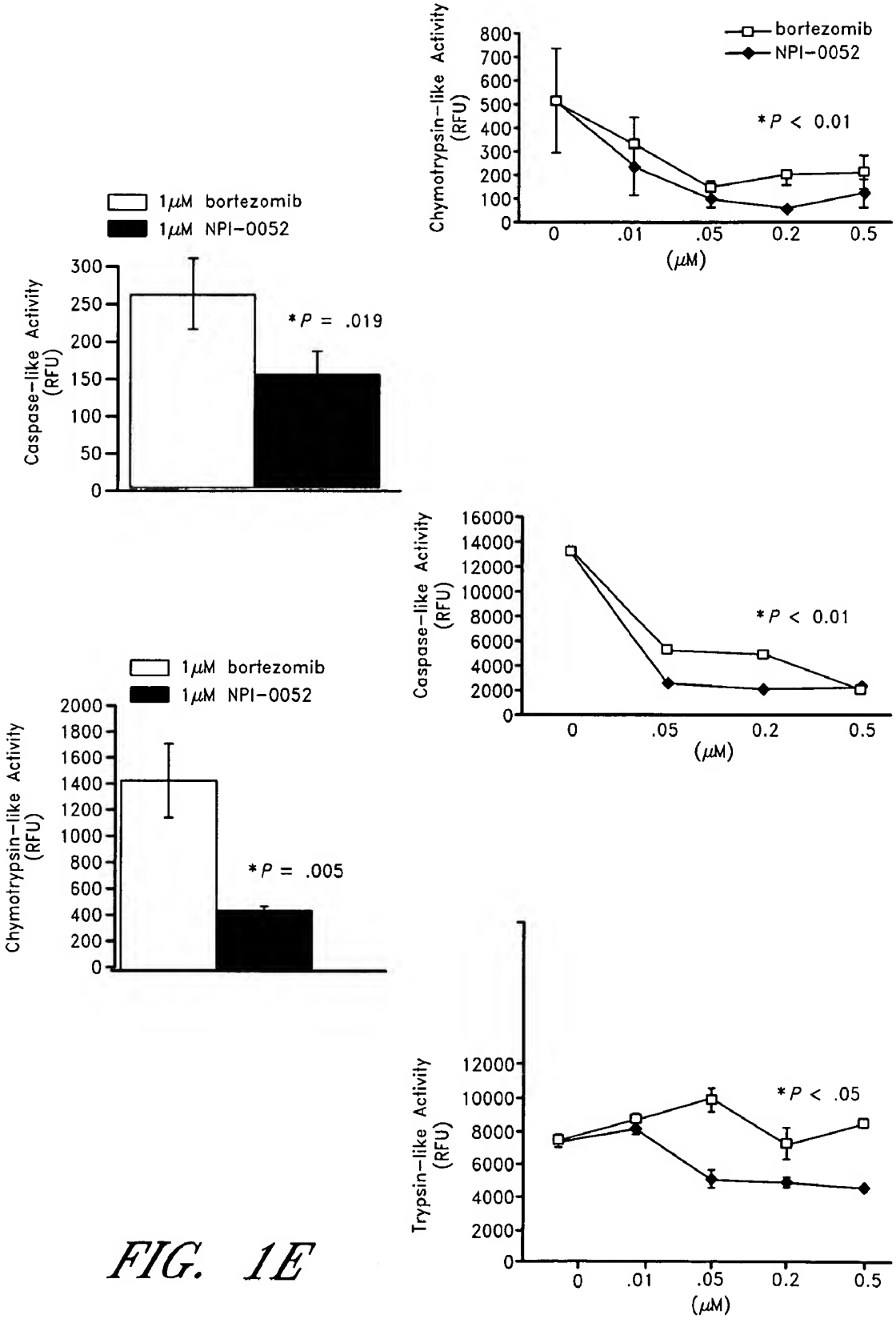

bortezomib
NPI-0052
Chymotrypsin-like Activity (RFU)
*P < 0.01
(μM)
1μM bortezomib
1μM NPI-0052
Caspase-like Activity (RFU)
*P = .019
Caspase-like Activity (RFU)
*P < 0.01
(μM)
1μM bortezomib
1μM NPI-0052
Chymotrypsin-like Activity (RFU)
*P = .005
Trypsin-like Activity (RFU)
*P < .05
(μM)

FIG. 1E

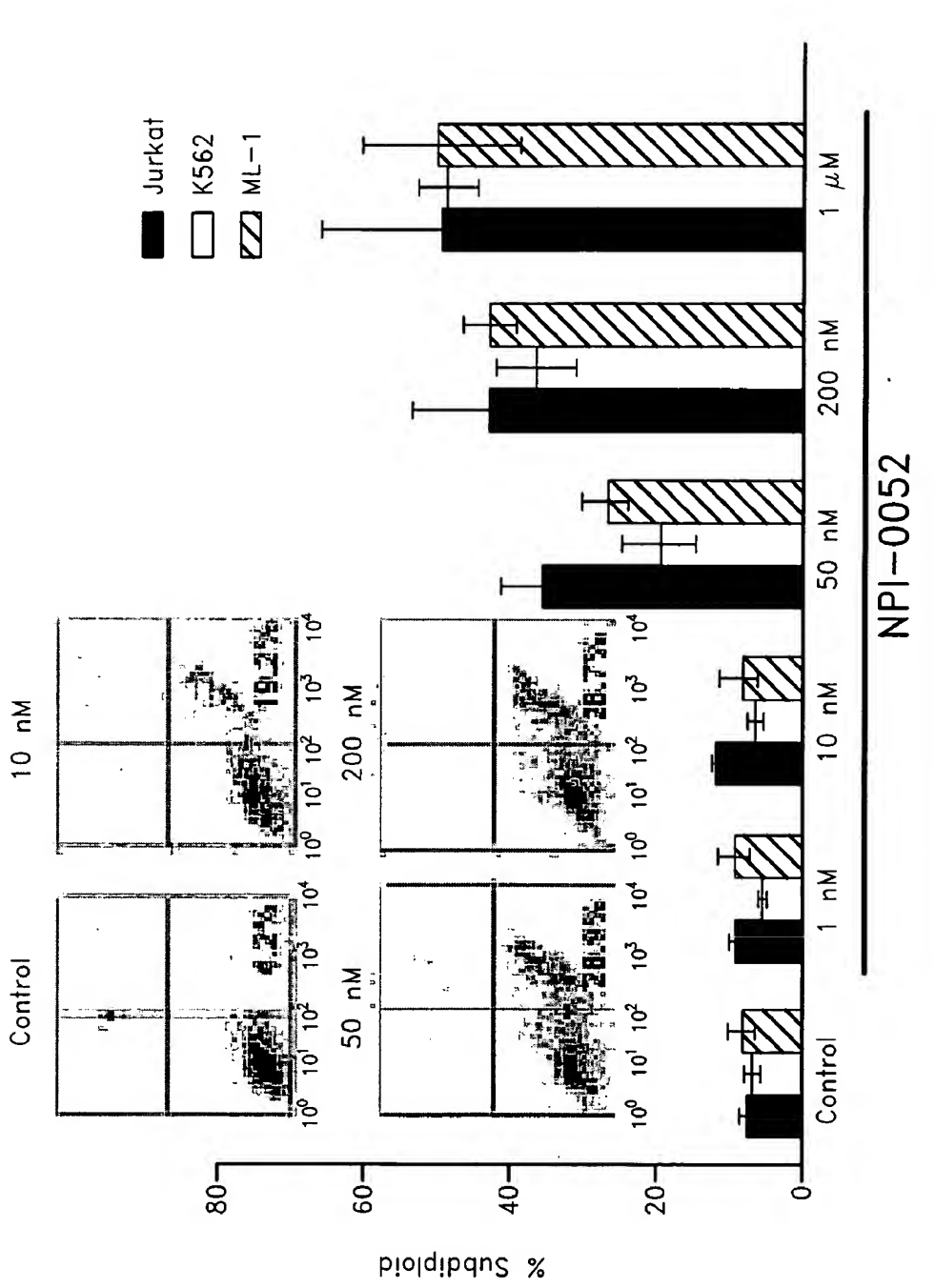

Jurkat
K562
ML-1
% Subdiploid
0
20
40
60
80
Control
1 nM
10 nM
50 nM
200 nM
1 μM
NPI-0052
Control
10 nM
50 nM
200 nM
19.2
28.8
28.7

FIG. 2A

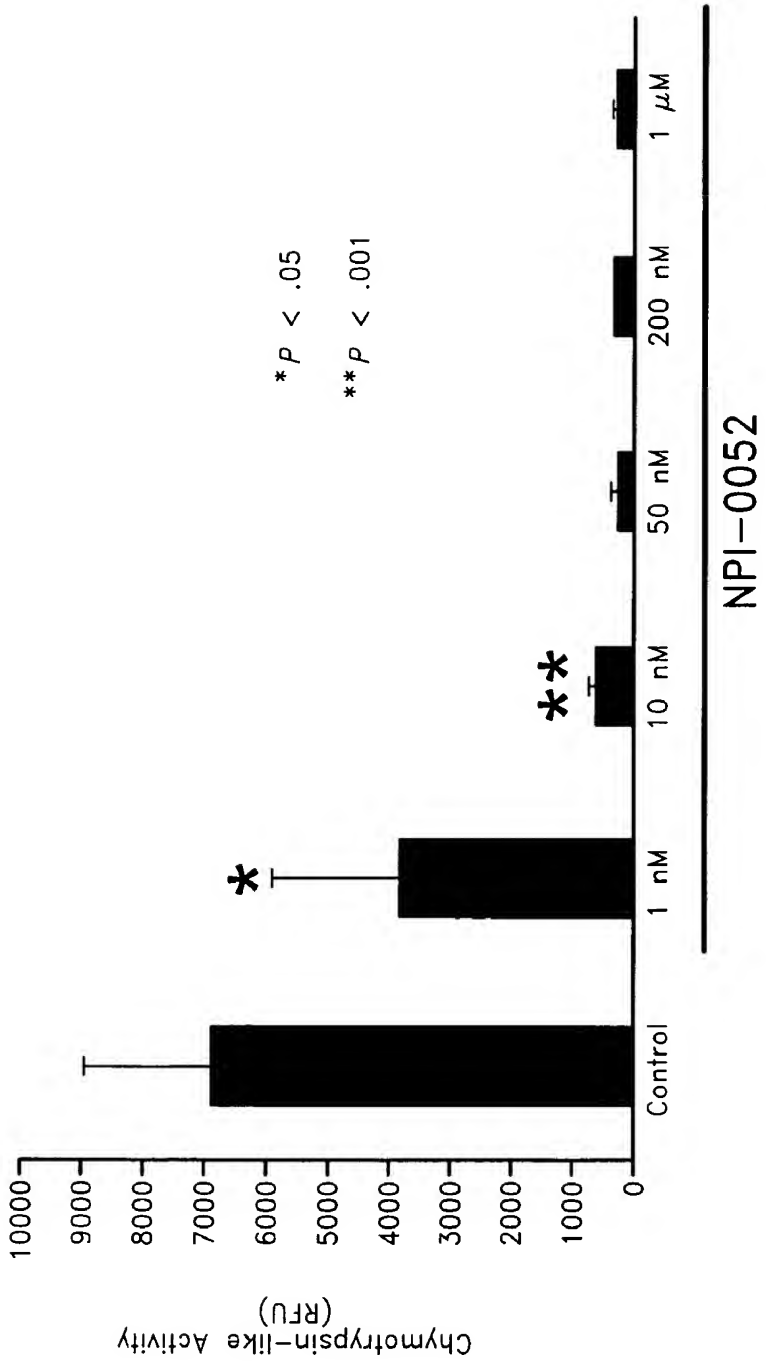

Chymotrypsin-like Activity
(RFU)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Control
1 nM
10 nM
50 nM
200 nM
1 μM
NPI-0052
*
**
*P < .05
**P < .001

FIG. 2B

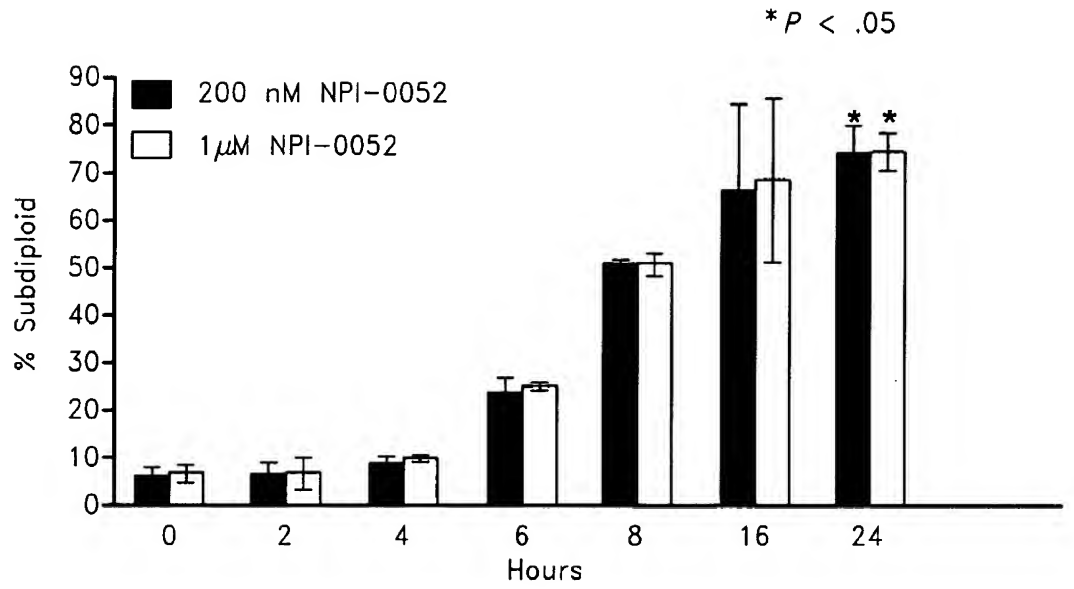

*P < .05
200 nM NPI-0052
1μM NPI-0052
% Subdiploid
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
16
24
Hours
*
*

FIG. 2C

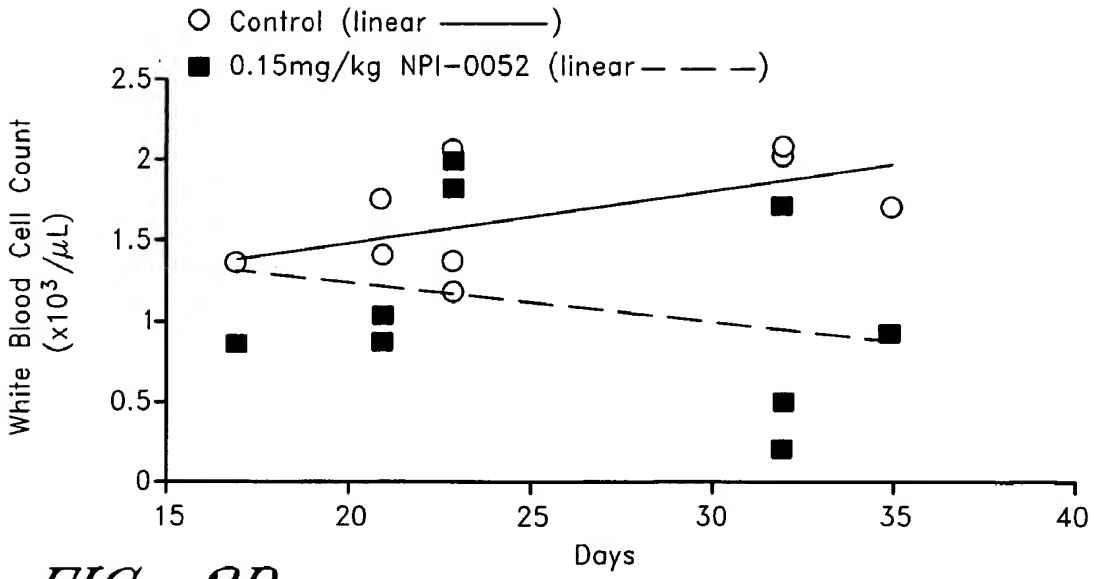

Control (linear ———)
0.15mg/kg NPI-0052 (linear — — —)
White Blood Cell Count (x10³/μL)
2.5
2
1.5
1
0.5
0
15
20
25
30
35
40
Days

FIG. 2D

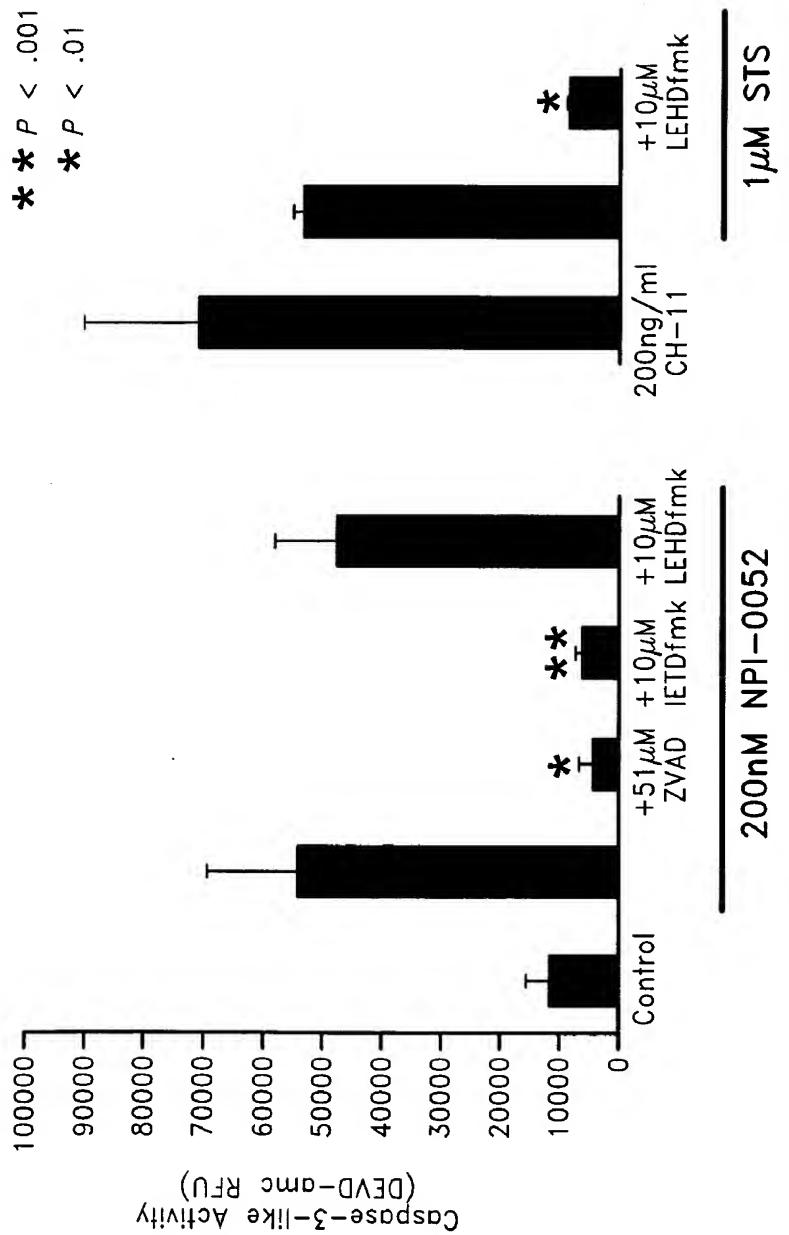

Caspase-3-like Activity
(DEVD-amc RFU)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Control
+51μM ZVAD
+10μM IETDfmk
+10μM LEHDfmk
200nM NPI-0052
200ng/ml CH-11
+10μM LEHDfmk
1μM STS
* P < .01
** P < .001

FIG. 3A

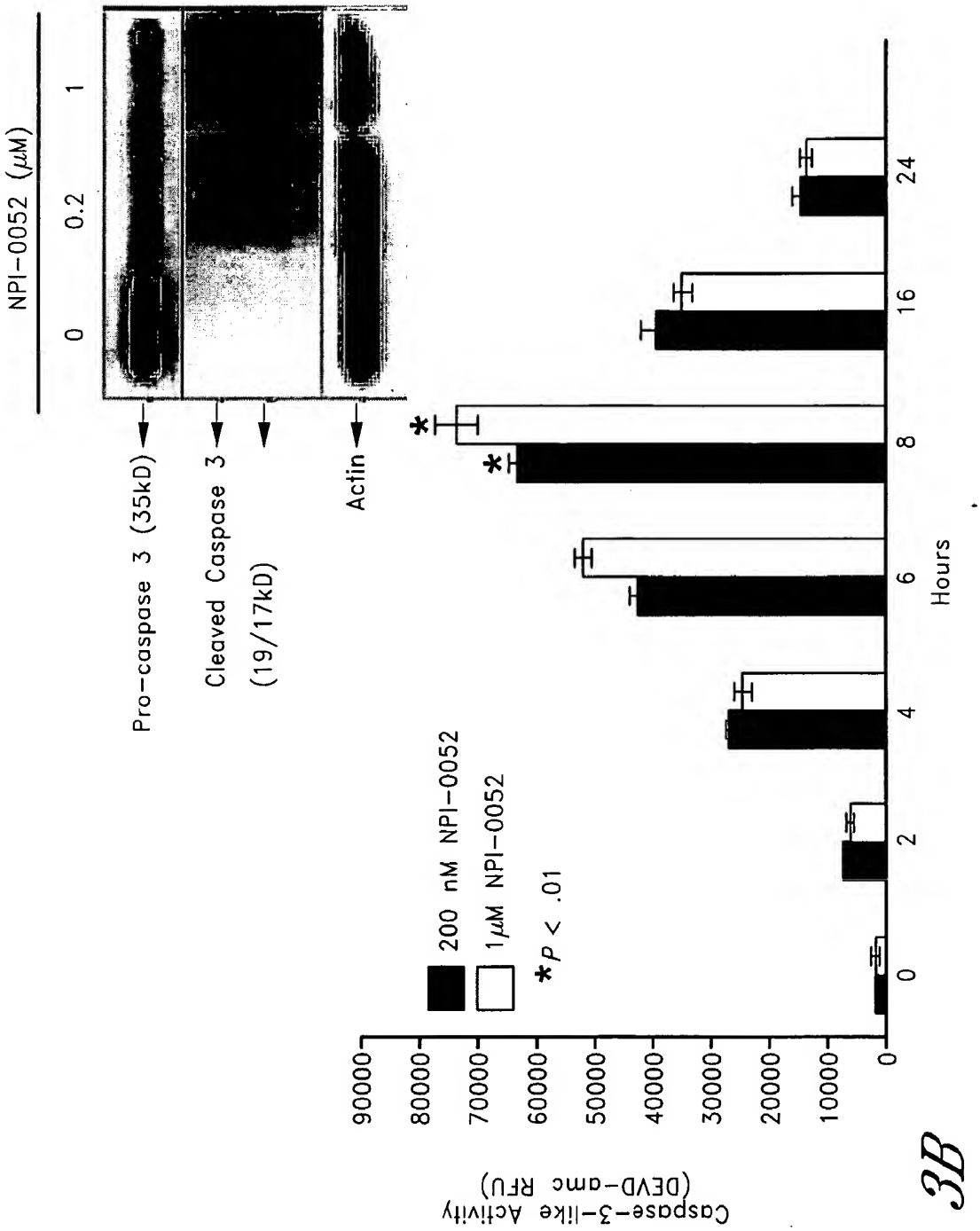

NPI-0052 (μM)
0
0.2
1
Pro-caspase 3 (35kD)
Cleaved Caspase 3
(19/17kD)
Actin
Caspase-3-like Activity
(DEVD-amc RFU)
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
200 nM NPI-0052
1μM NPI-0052
*P < .01
0
2
4
6
8
16
24
Hours

FIG. 3B

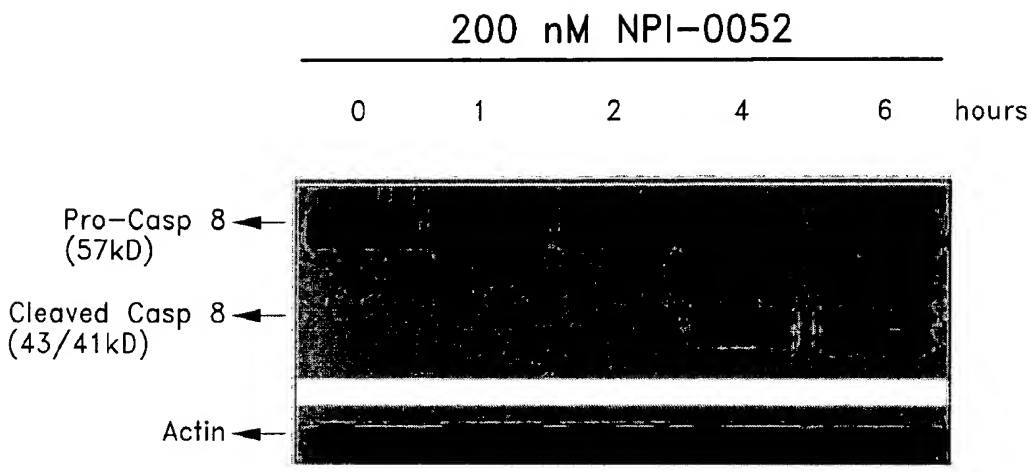


FIG. 3C

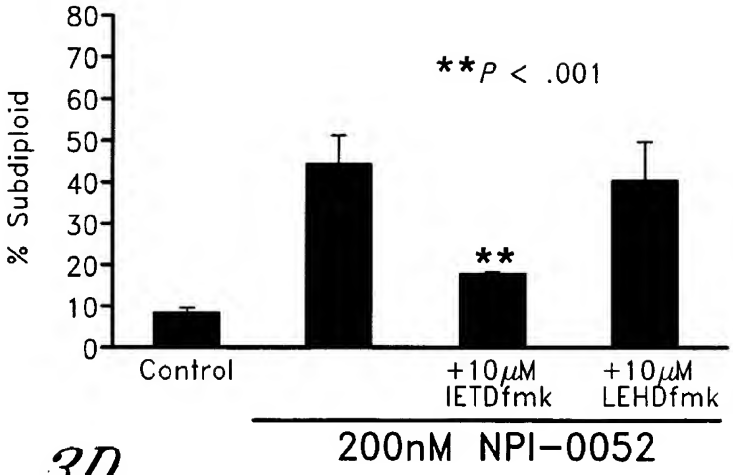


FIG. 3D

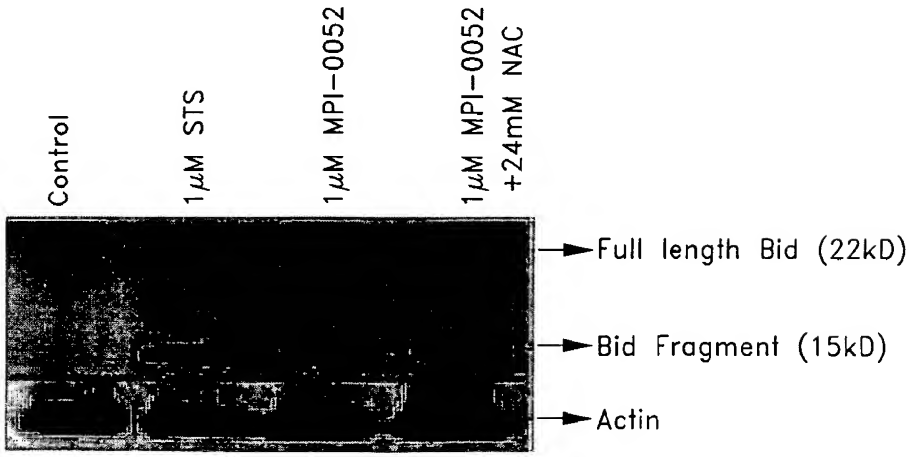


FIG. 3D

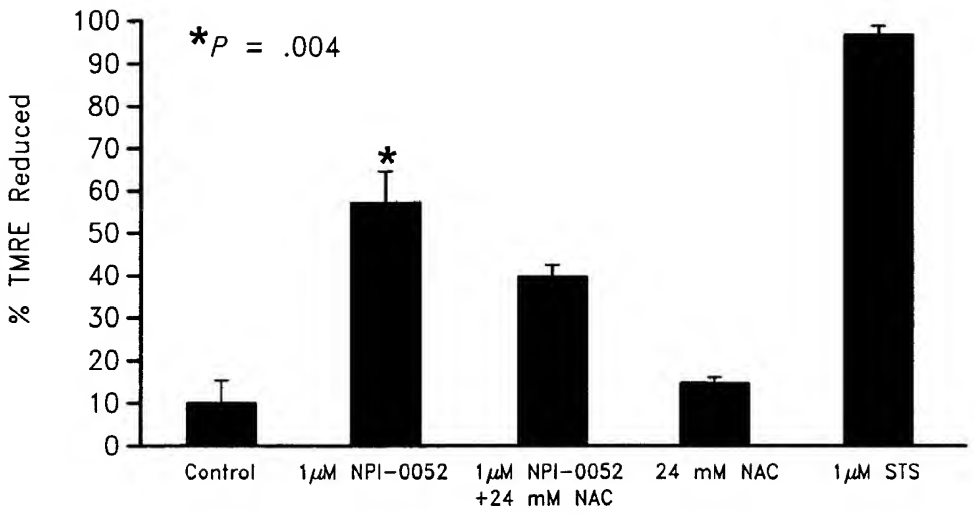

*P = .004
% TMRE Reduced
100
90
80
70
60
50
40
30
20
10
0
Control
1μM NPI-0052
1μM NPI-0052 +24 mM NAC
24 mM NAC
1μM STS

FIG. 3F

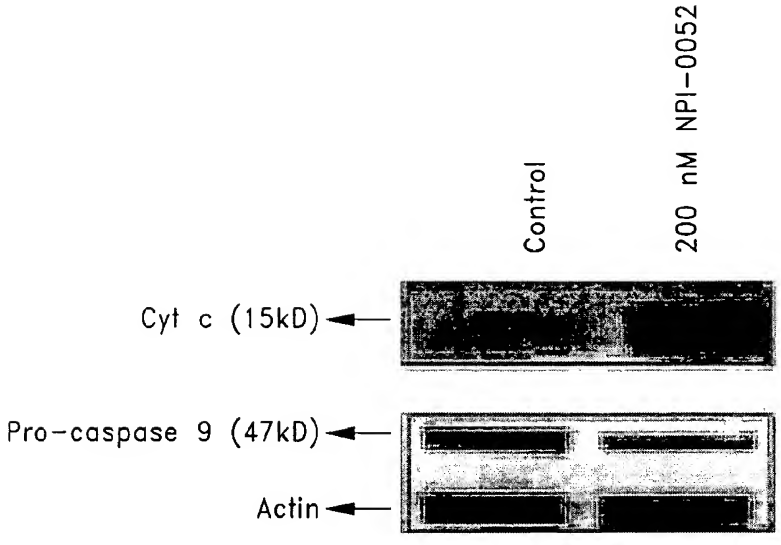

Control
200 nM NPI-0052
Cyt c (15kD)
Pro-caspase 9 (47kD)
Actin

FIG. 3G

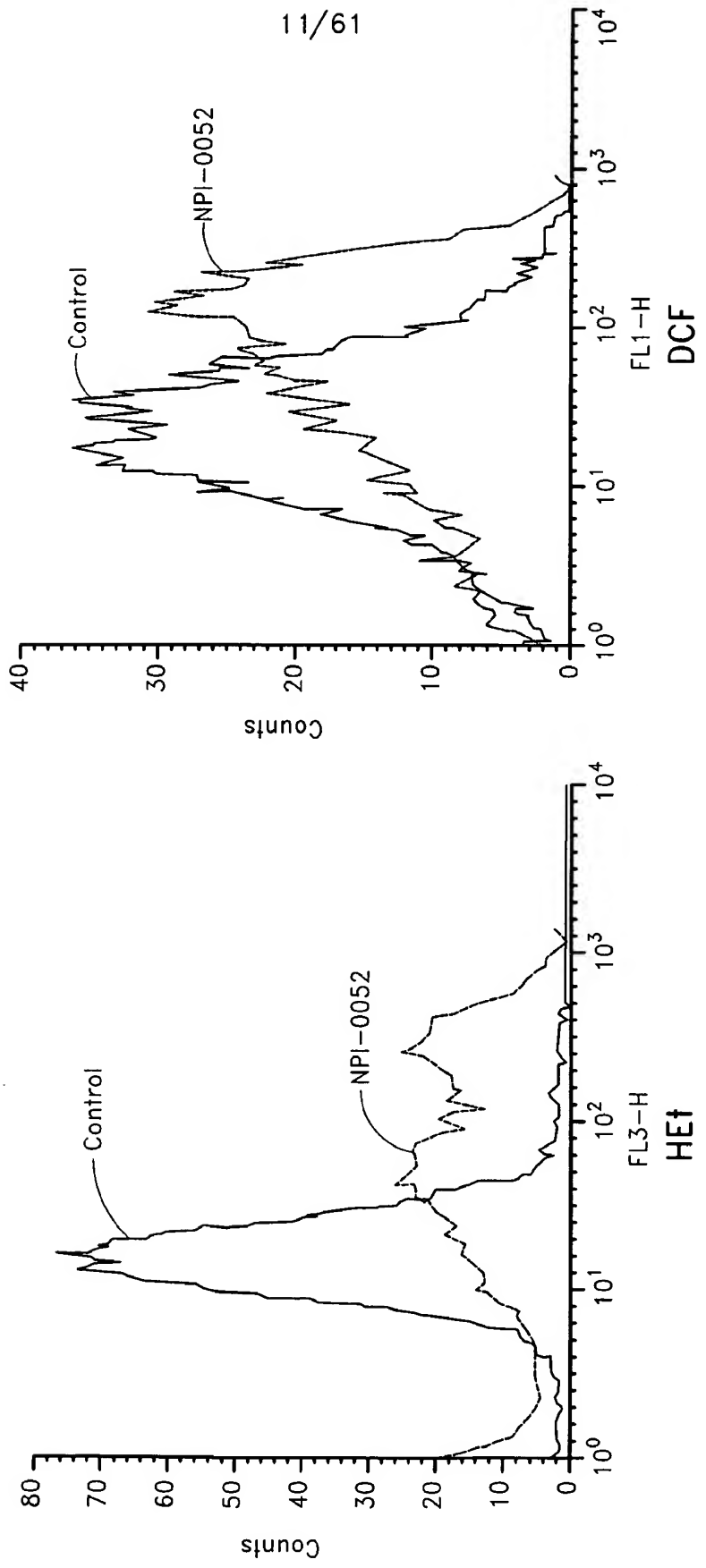

Control
NPI-0052
Counts
FL1-H
DCF
Control
NPI-0052
Counts
FL3-H
HEt

FIG. 3H

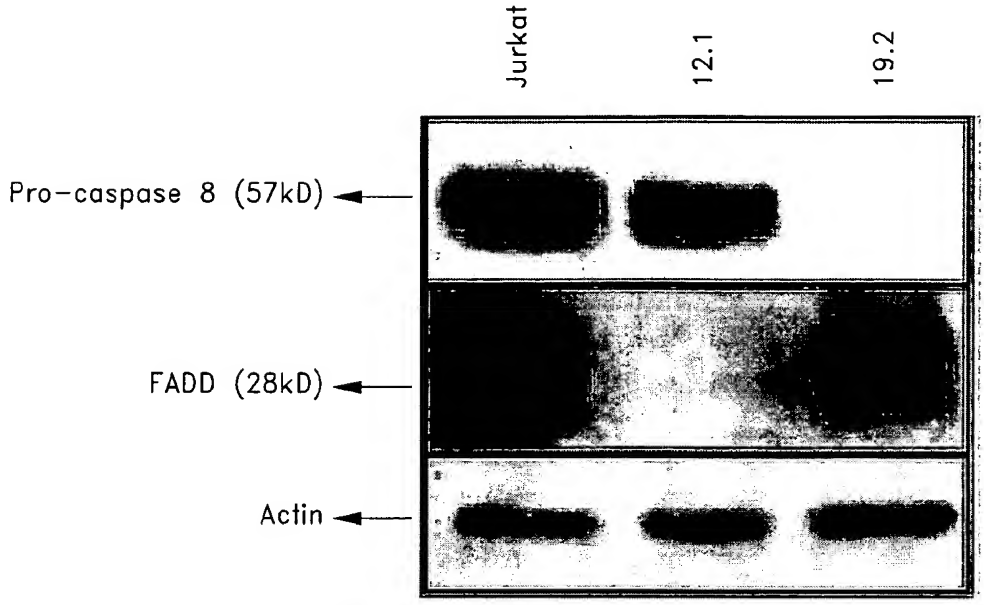

Jurkat
12.1
19.2
Pro-caspase 8 (57kD)
FADD (28kD)
Actin

FIG. 4A

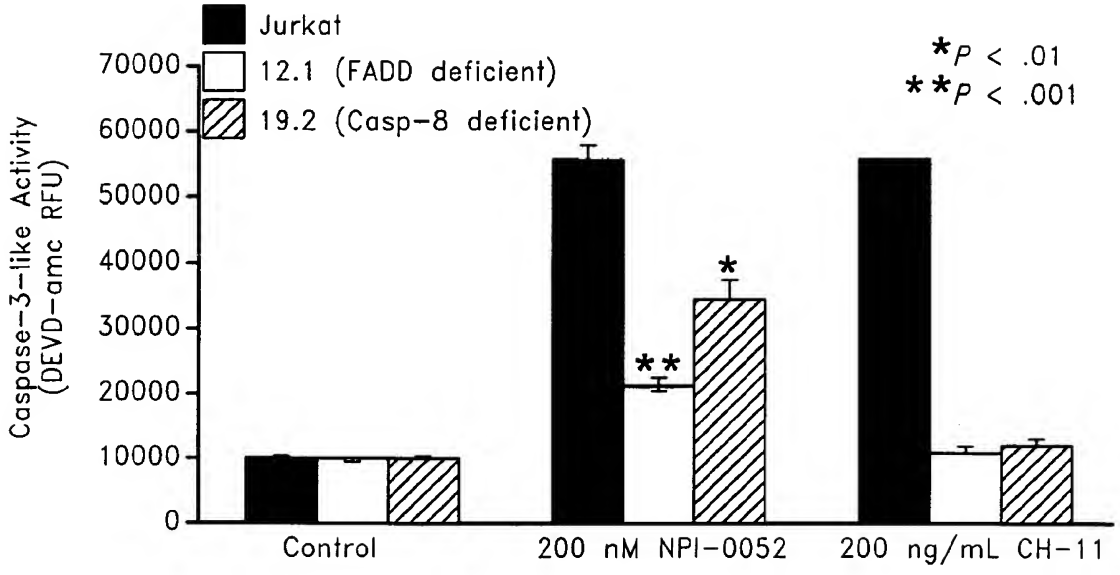

Jurkat
12.1 (FADD deficient)
19.2 (Casp-8 deficient)
*P < .01
**P < .001
Caspase-3-like Activity (DEVD-amc RFU)
70000
60000
50000
40000
30000
20000
10000
0
**
*
Control
200 nM NPI-0052
200 ng/mL CH-11

FIG. 4B

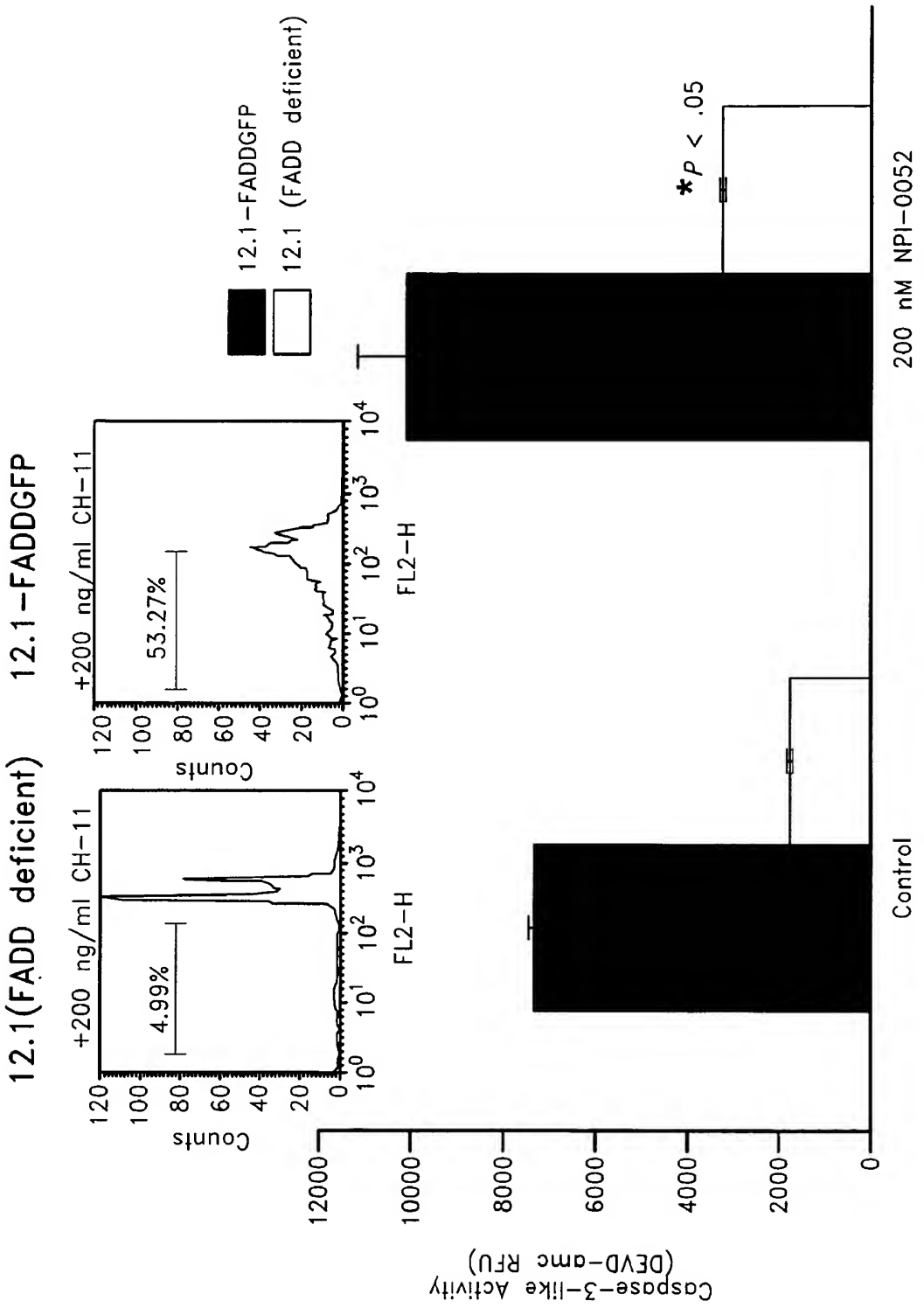

12.1(FADD deficient)
12.1-FADDGFP
+200 ng/ml CH-11
4.99%
+200 ng/ml CH-11
53.27%
Counts
FL2-H
12.1-FADDGFP
12.1 (FADD deficient)
Caspase-3-like Activity
(DEVD-amc RFU)
*P < .05
Control
200 nM NPI-0052

FIG. 4C

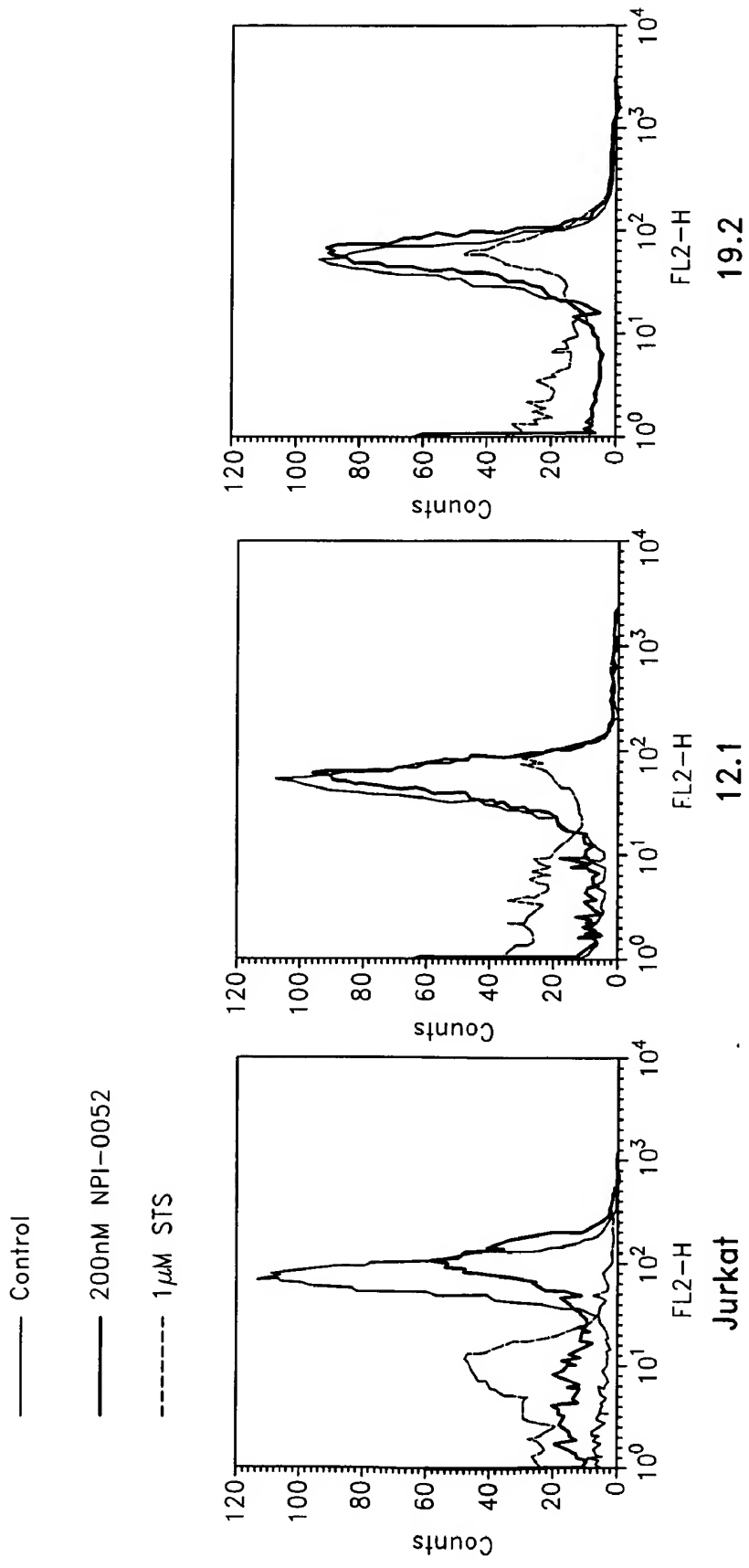


FIG. 4D

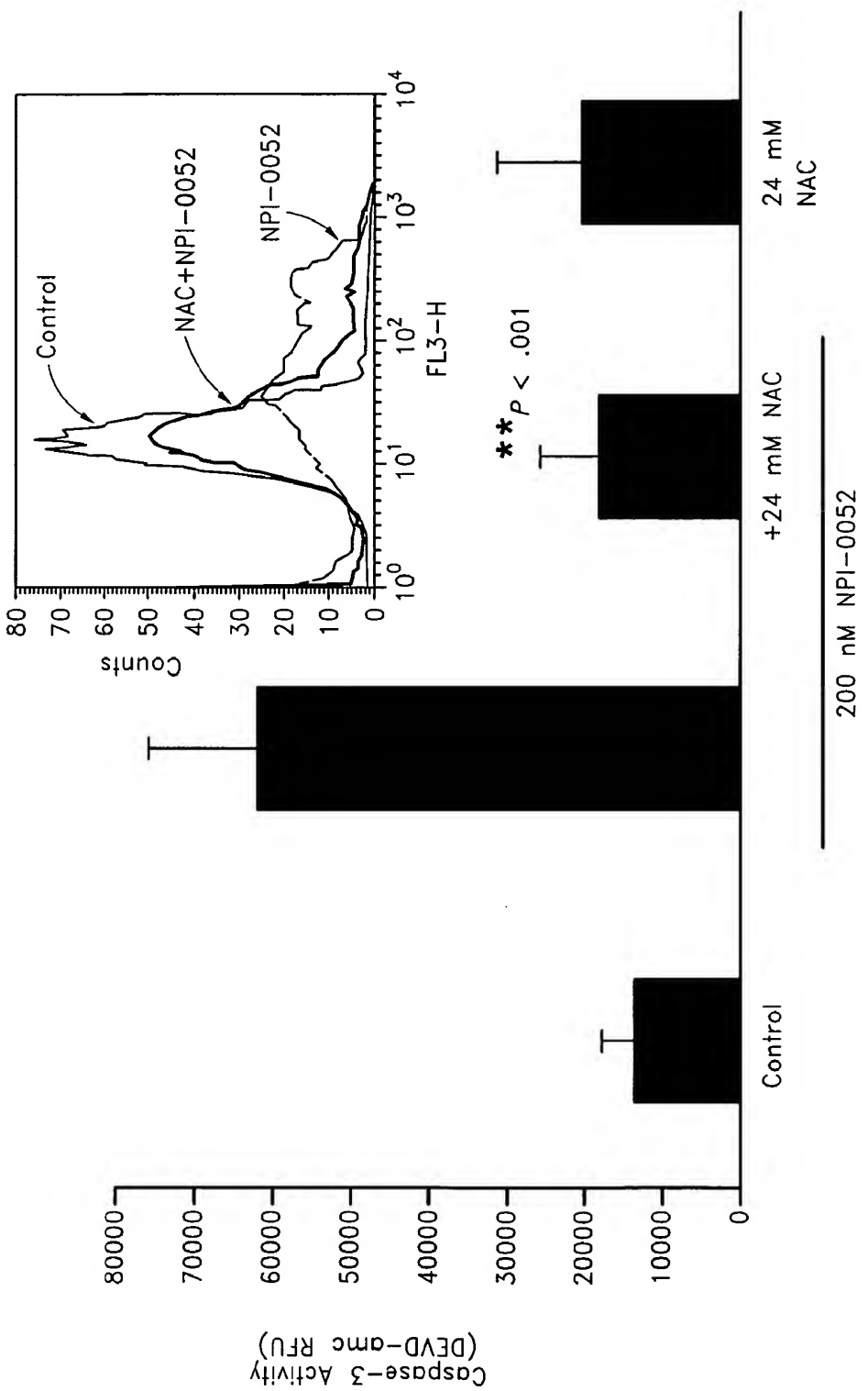

Caspase-3 Activity
(DEVD-amc RFU)
80000
70000
60000
50000
40000
30000
20000
10000
0
Control
+24 mM NAC
24 mM NAC
200 nM NPI-0052
**P < .001
Counts
80
70
60
50
40
30
20
10
0
Control
NAC+NPI-0052
NPI-0052
10^0
10^1
10^2
10^3
10^4
FL3-H

FIG. 5A

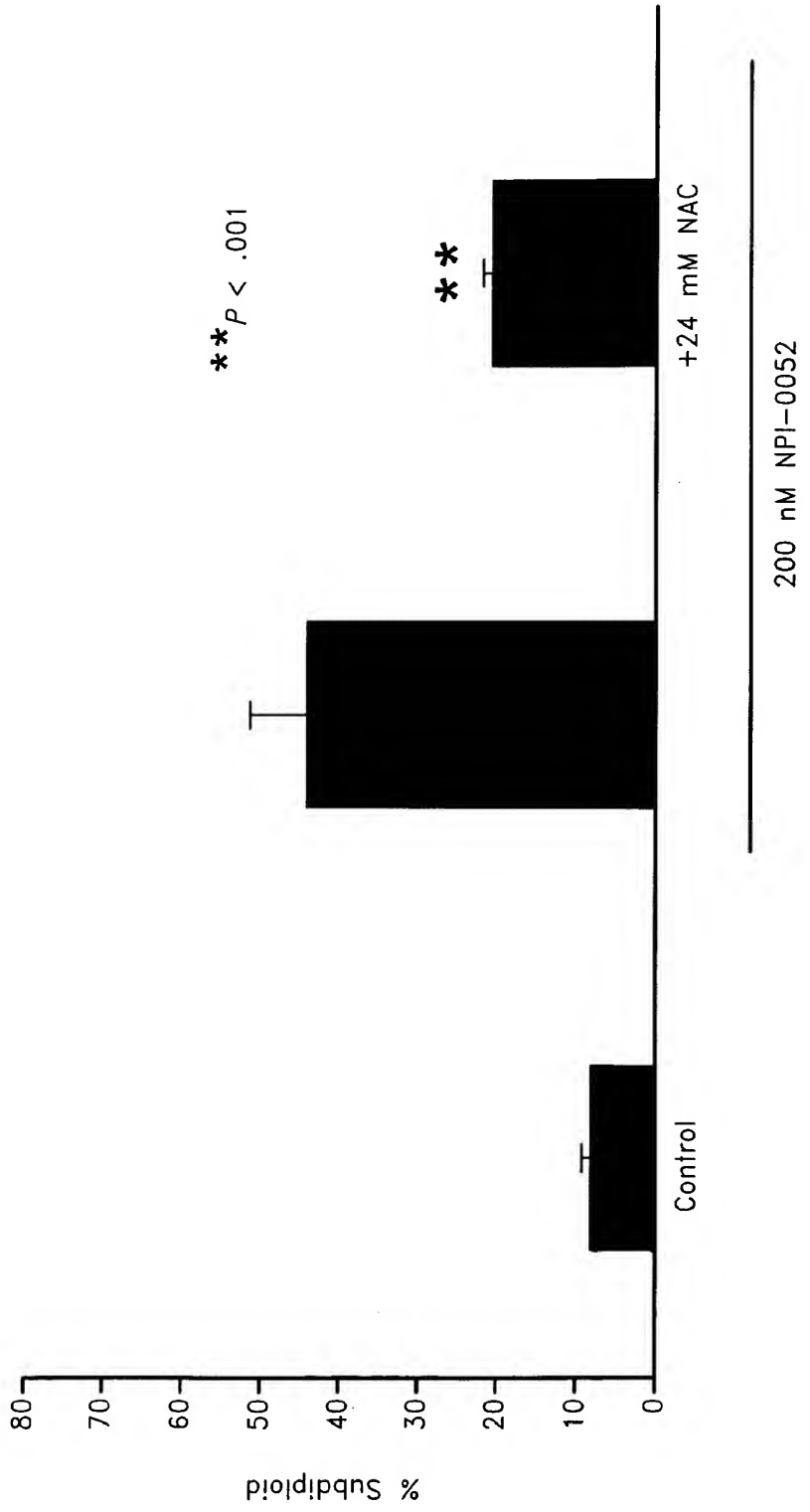

% Subdiploid
0
10
20
30
40
50
60
70
80
Control
+24 mM NAC
200 nM NPI-0052
**
**P < .001

FIG. 5B

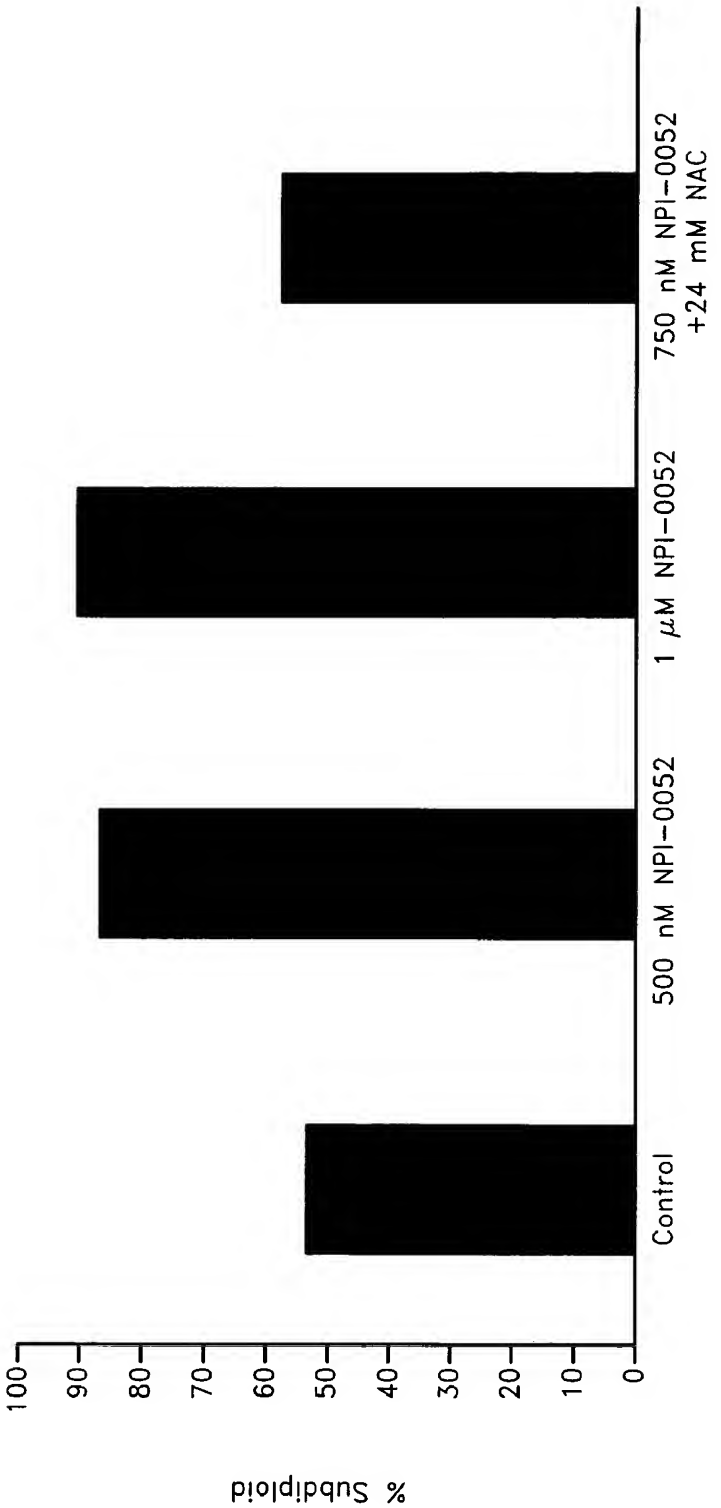
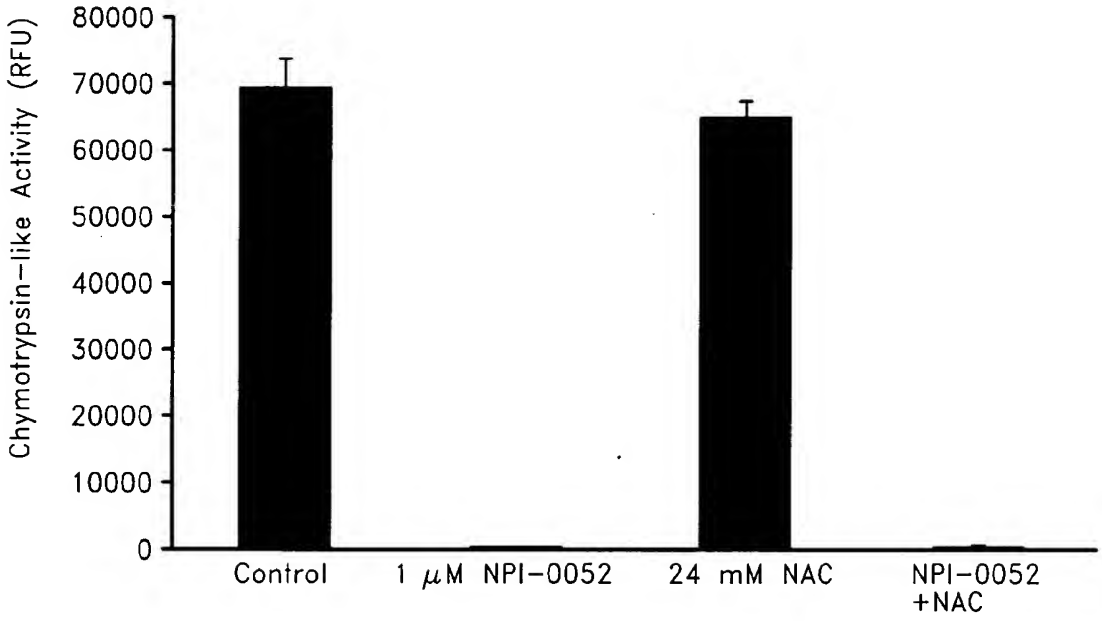

% Subdiploid
100
90
80
70
60
50
40
30
20
10
0
Control
500 nM NPI-0052
1 μM NPI-0052
750 nM NPI-0052
+24 mM NAC

FIG. 5C


Chymotrypsin-like Activity (RFU)
80000
70000
60000
50000
40000
30000
20000
10000
0
Control
1 μM NPI-0052
24 mM NAC
NPI-0052 +NAC

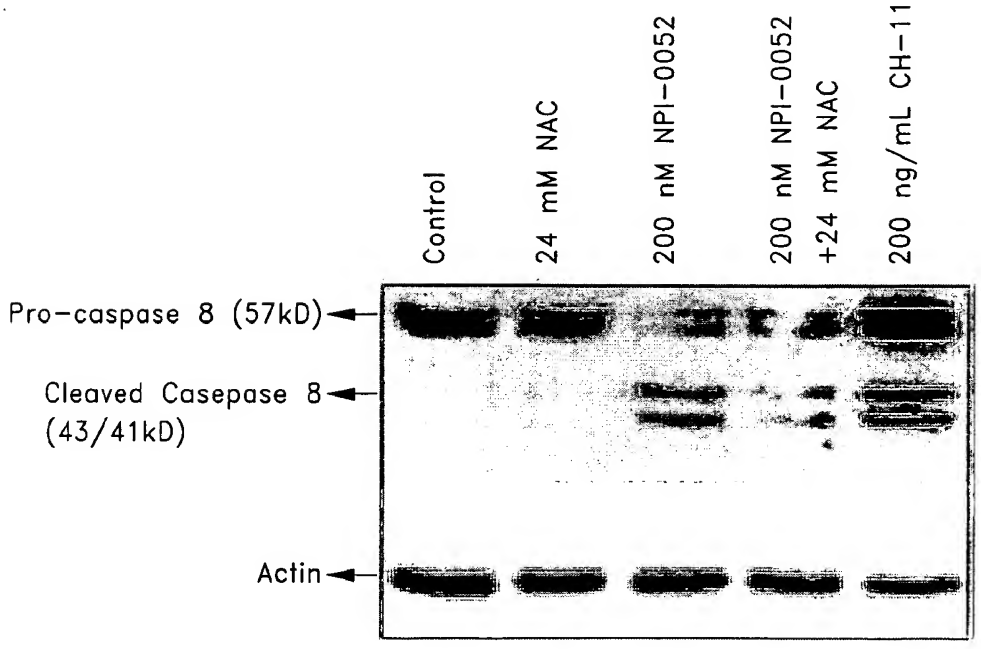

Control
24 mM NAC
200 nM NPI-0052
200 nM NPI-0052 +24 mM NAC
200 ng/mL CH-11
Pro-caspase 8 (57kD)
Cleaved Casepase 8 (43/41kD)
Actin

FIG. 5E

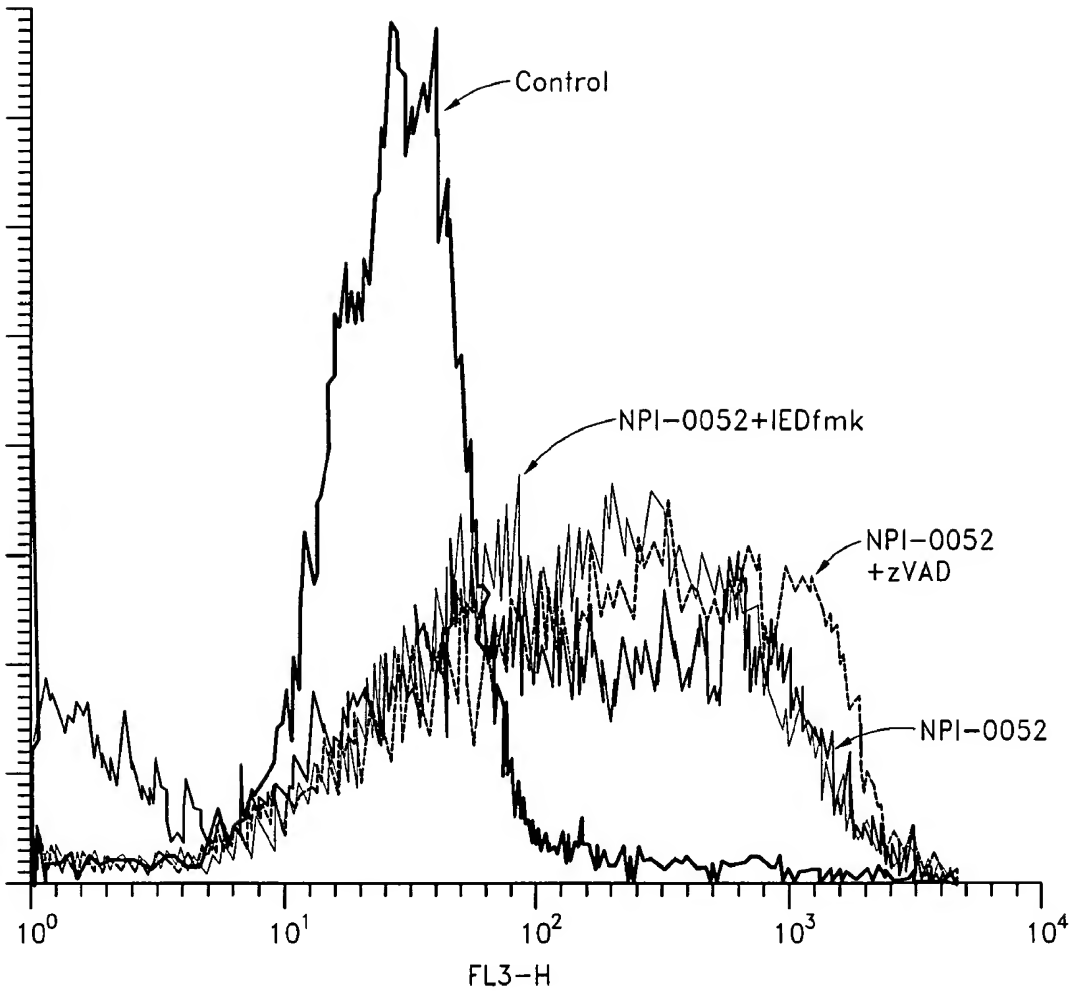

Control
NPI-0052+IEDfmk
NPI-0052
+zVAD
NPI-0052
10^0
10^1
10^2
10^3
10^4
FL3-H

FIG. 5F

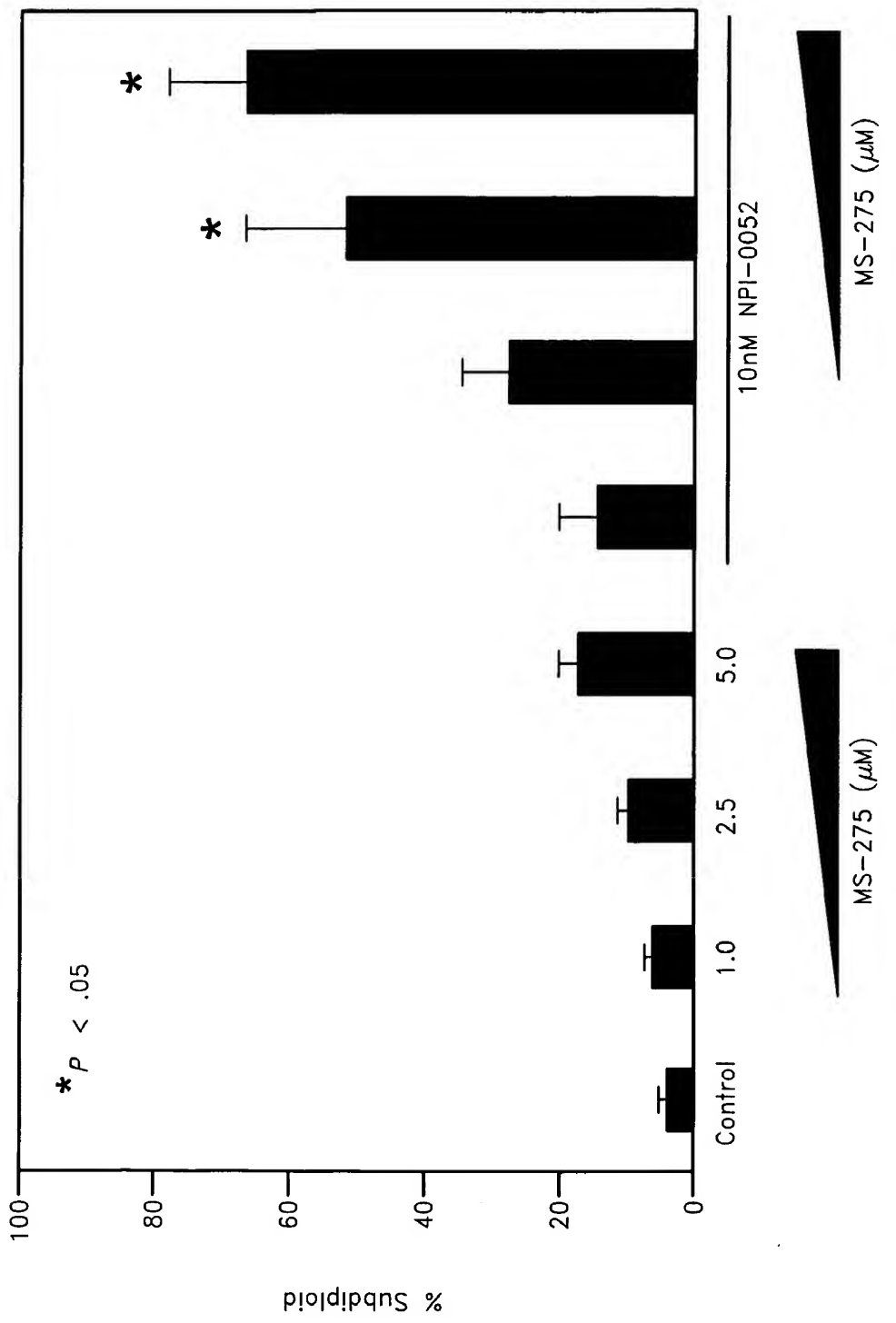

% Subdiploid
100
80
60
40
20
0
*P < .05
Control
1.0
2.5
5.0
MS-275 (μM)
10nM NPI-0052
MS-275 (μM)

FIG. 6A

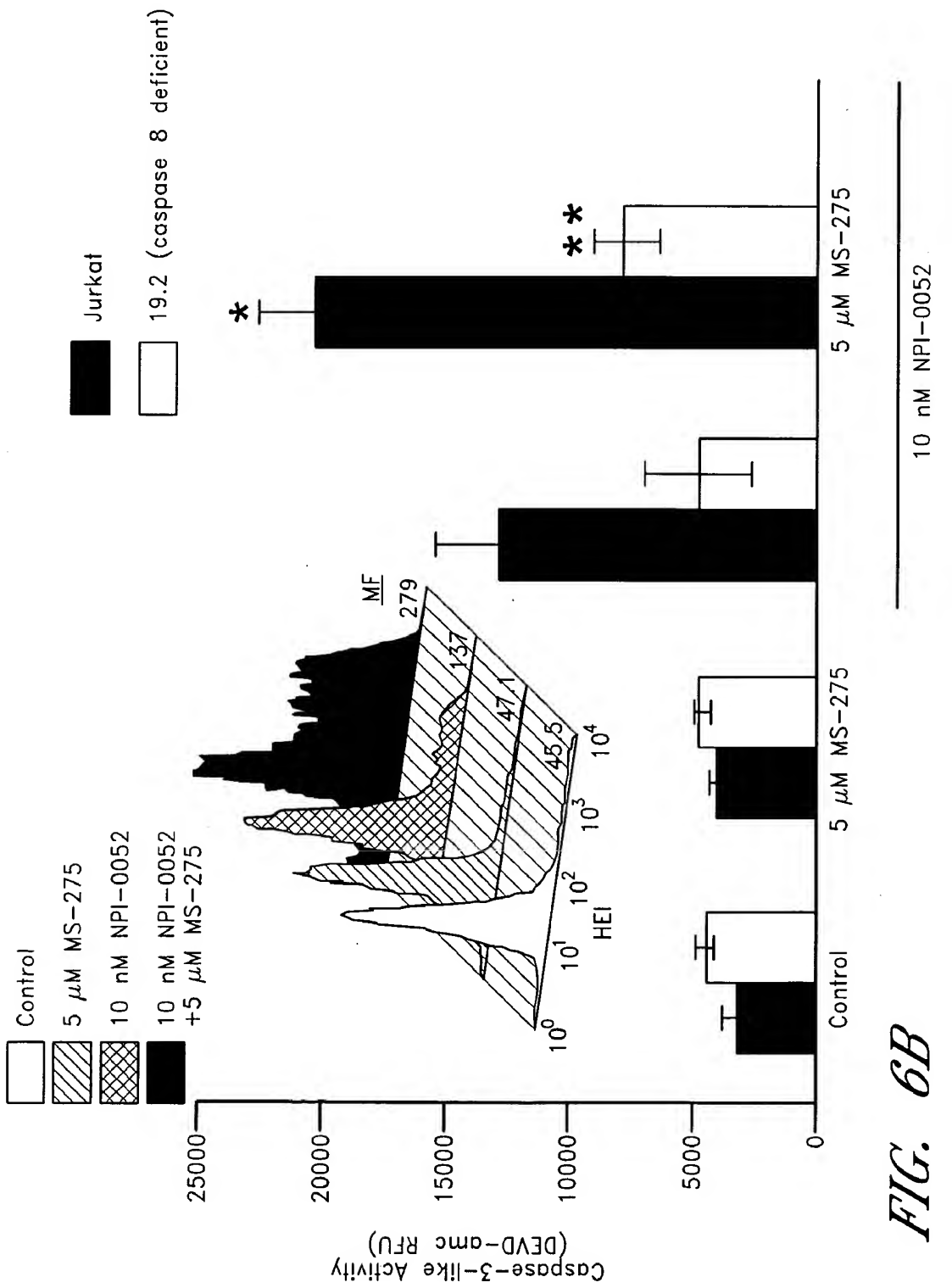

Caspase-3-like Activity
(DEVD-amc RFU)
25000
20000
15000
10000
5000
0
Control
5 μM MS-275
10 nM NPI-0052
10 nM NPI-0052
+5 μM MS-275
Jurkat
19.2 (caspase 8 deficient)
*
**
Control
5 μM MS-275
5 μM MS-275
10 nM NPI-0052
MF
279
137
47.1
45.5
HEI
10^0
10^1
10^2
10^3
10^4

FIG. 6B

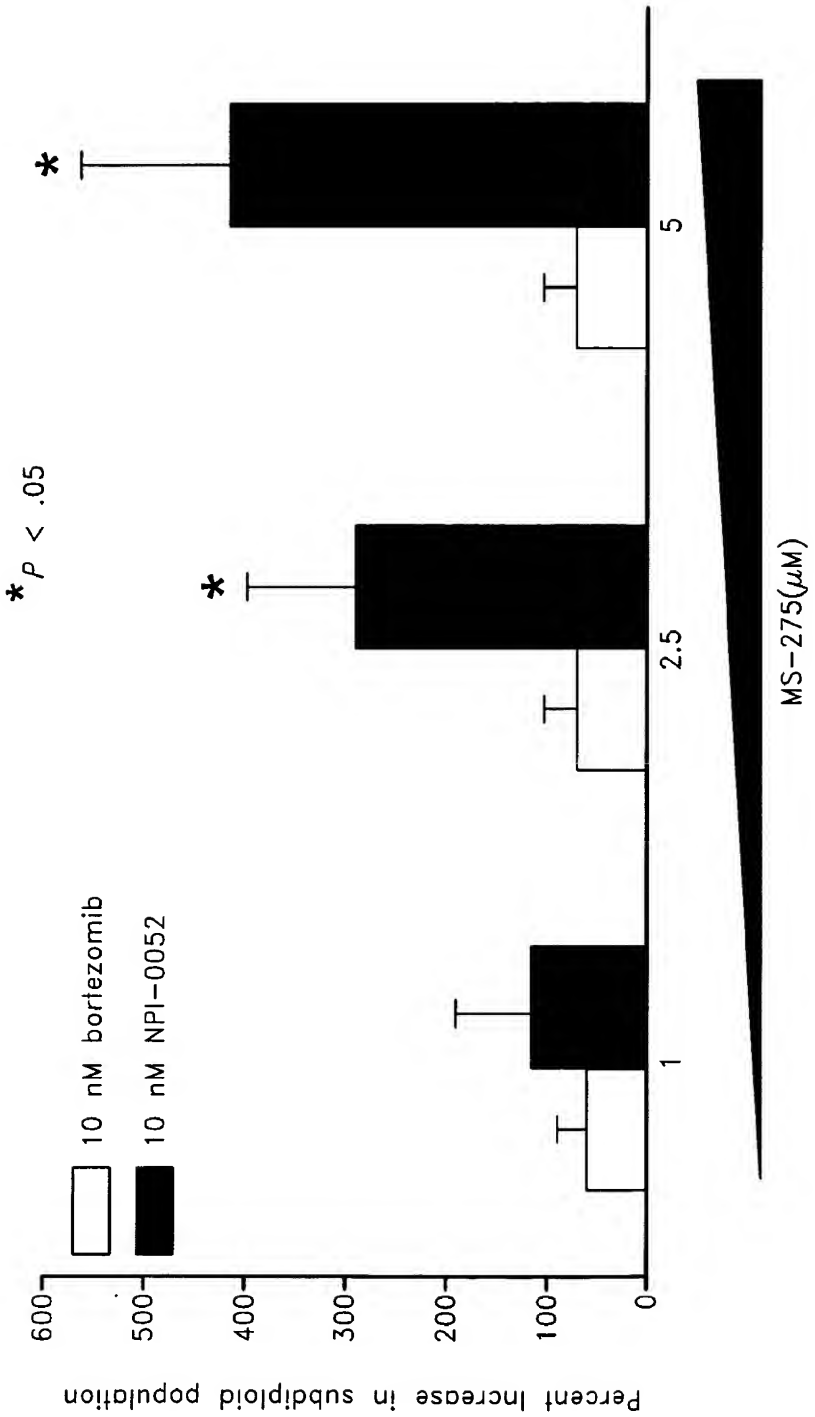

Percent Increase in subdiploid population
600
500
400
300
200
100
0
10 nM bortezomib
10 nM NPI-0052
*p < .05
*
*
1
2.5
5
MS-275(μM)

FIG. 6C

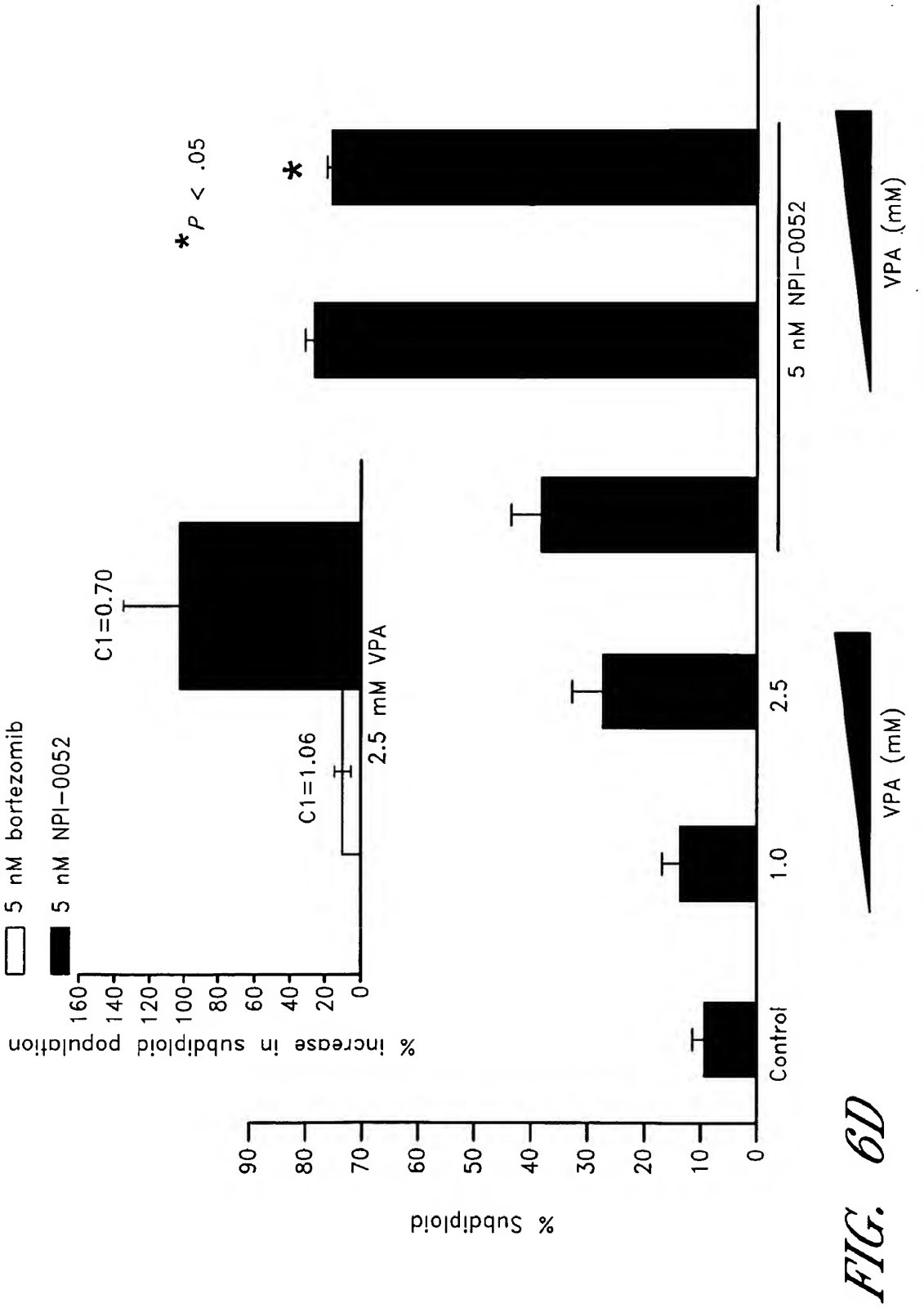

% Subdiploid
90
80
70
60
50
40
30
20
10
0
Control
1.0
2.5
5 nM NPI-0052
VPA (mM)
VPA (mM)
*
*P < .05
% increase in subdiploid population
160
140
120
100
80
60
40
20
0
5 nM bortezomib
5 nM NPI-0052
C1=0.70
C1=1.06
2.5 mM VPA

FIG. 6D

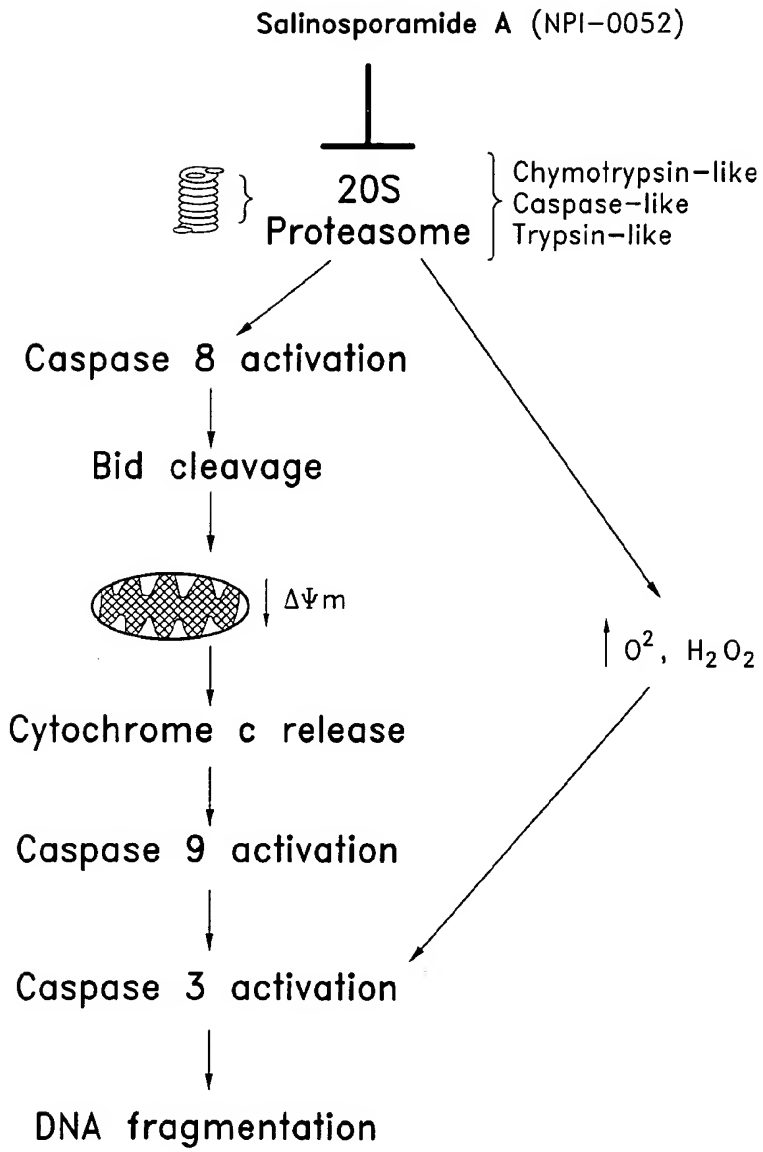

Salinosporamide A (NPI-0052)
20S Proteasome
Chymotrypsin-like
Caspase-like
Trypsin-like
Caspase 8 activation
Bid cleavage
ΔΨm
Cytochrome c release
Caspase 9 activation
Caspase 3 activation
DNA fragmentation
O2, H2O2

FIG. 7

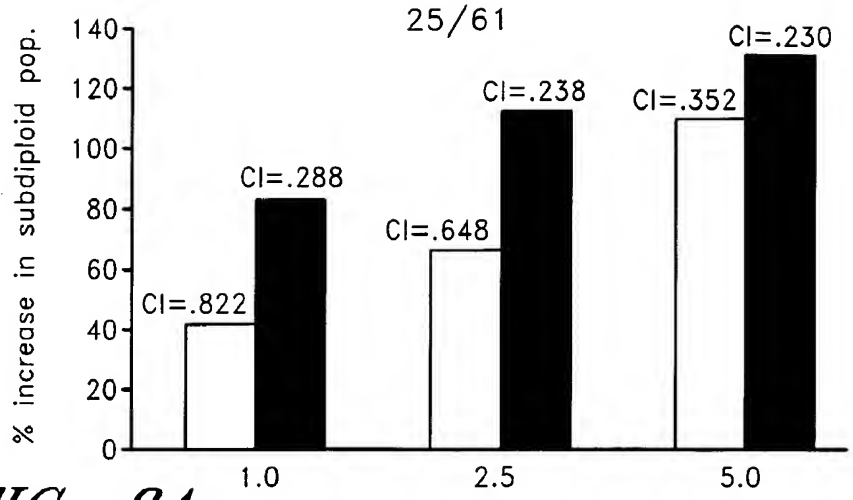


FIG. 8A

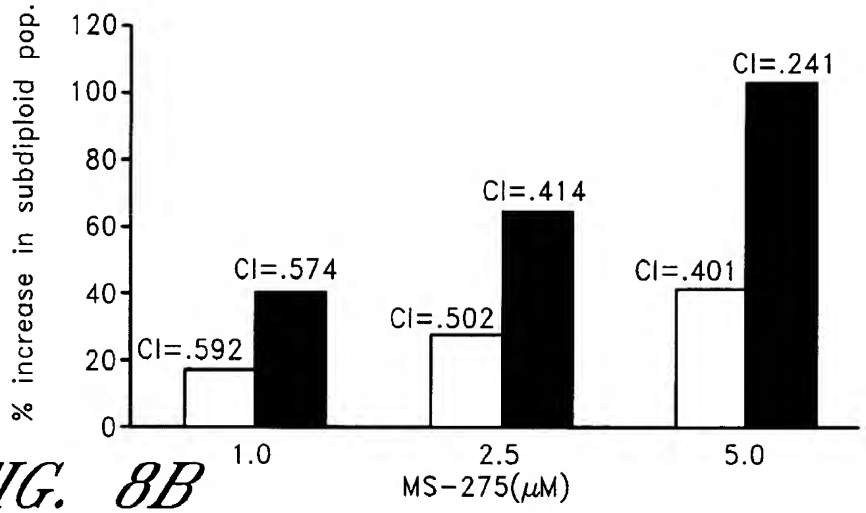


FIG. 8B

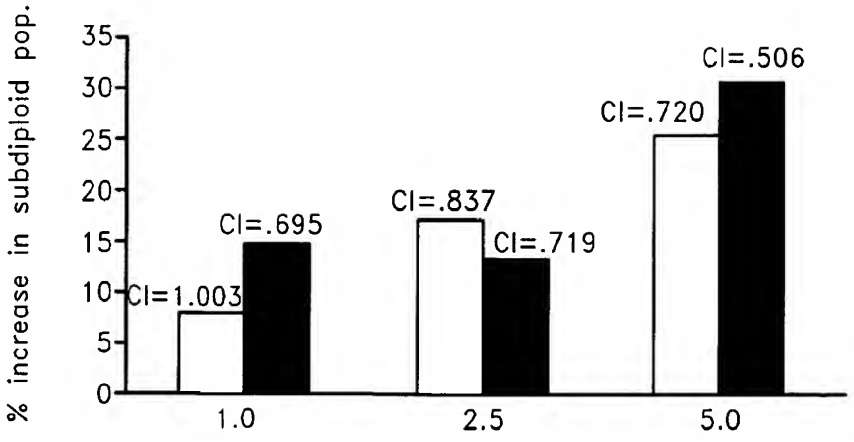


FIG. 8C

A-1nm B-5nM C-10nM
Synergy combination index (CI)<1
Additive effect CI=1
Antagonism CI>1

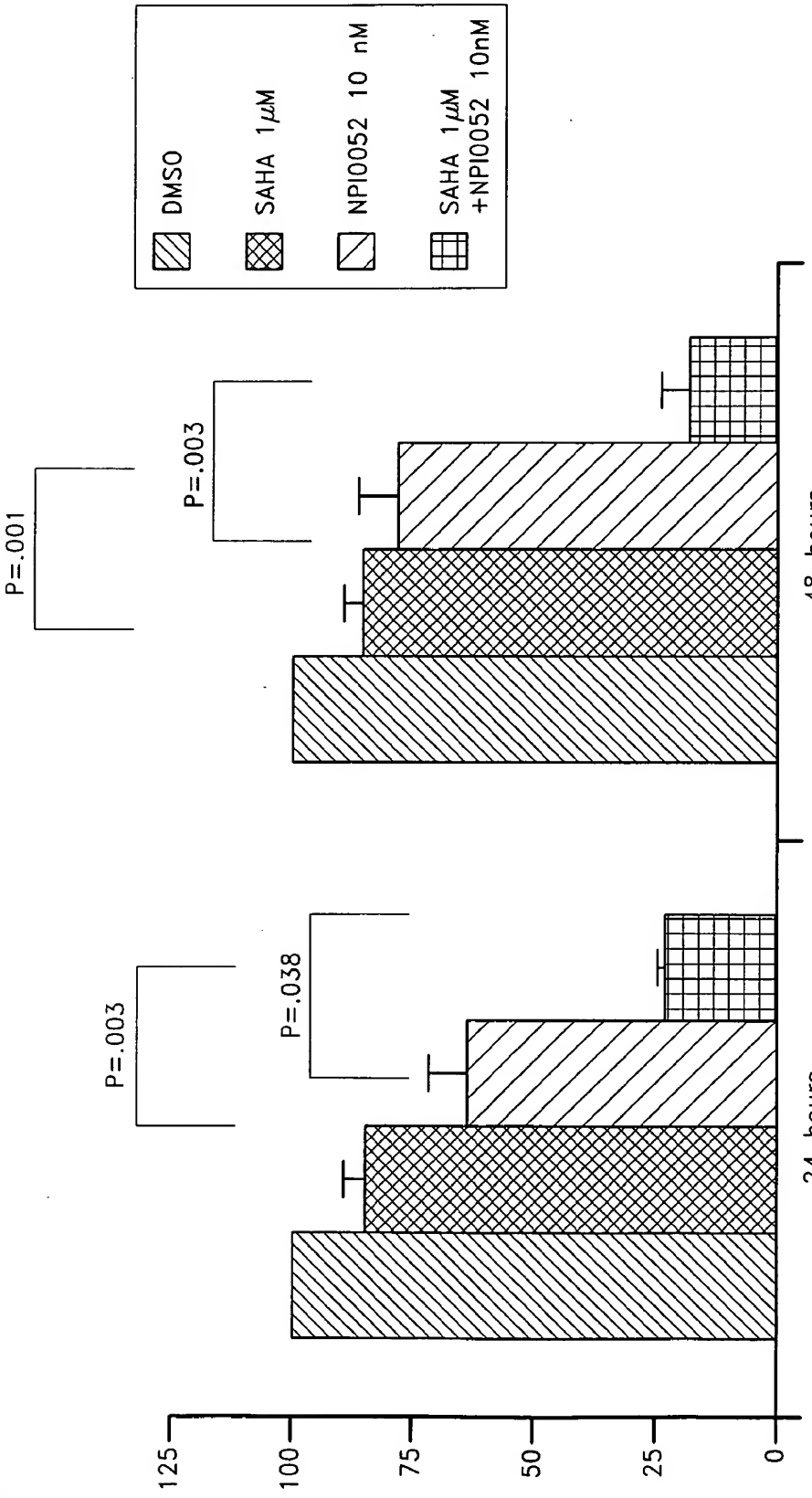

DMSO
SAHA 1μM
NPI0052 10 nM
SAHA 1μM +NPI0052 10nM
P=.001
P=.003
P=.003
P=.038
48 hours
24 hours
125
100
75
50
25
0

FIG. 9

FIG. 10A

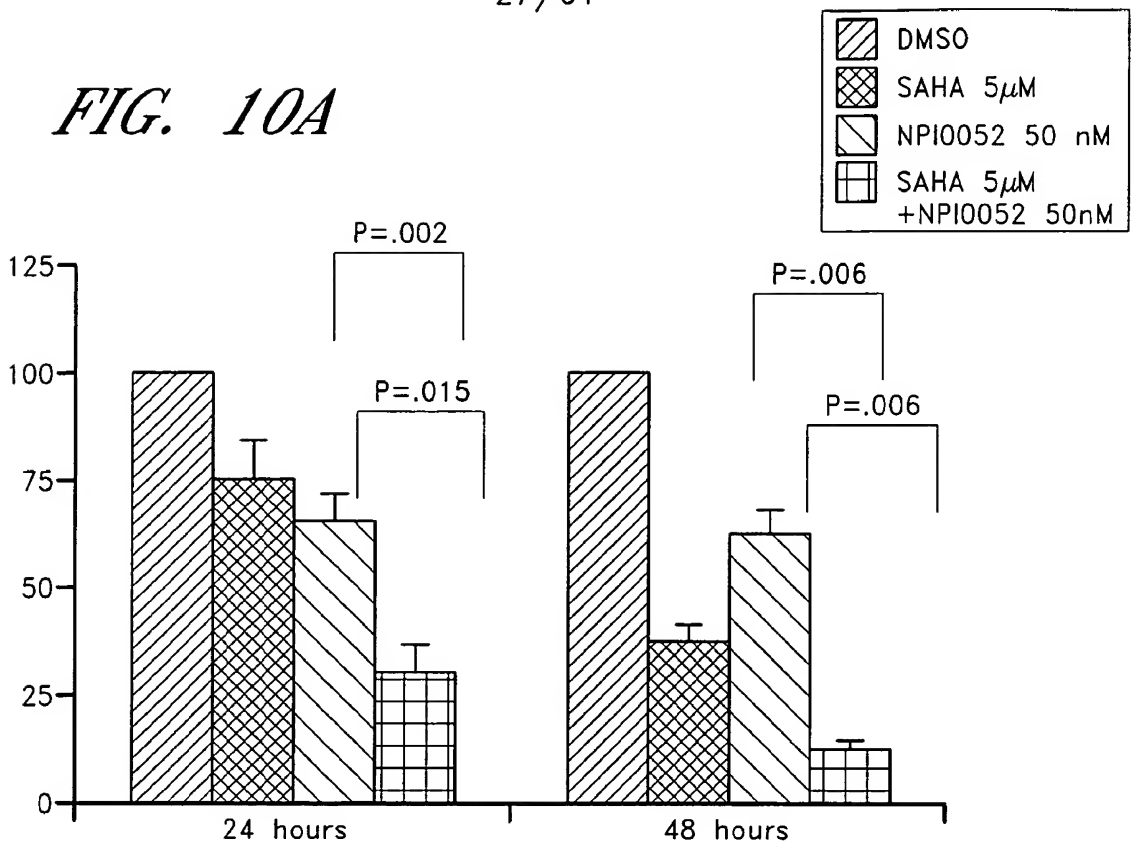
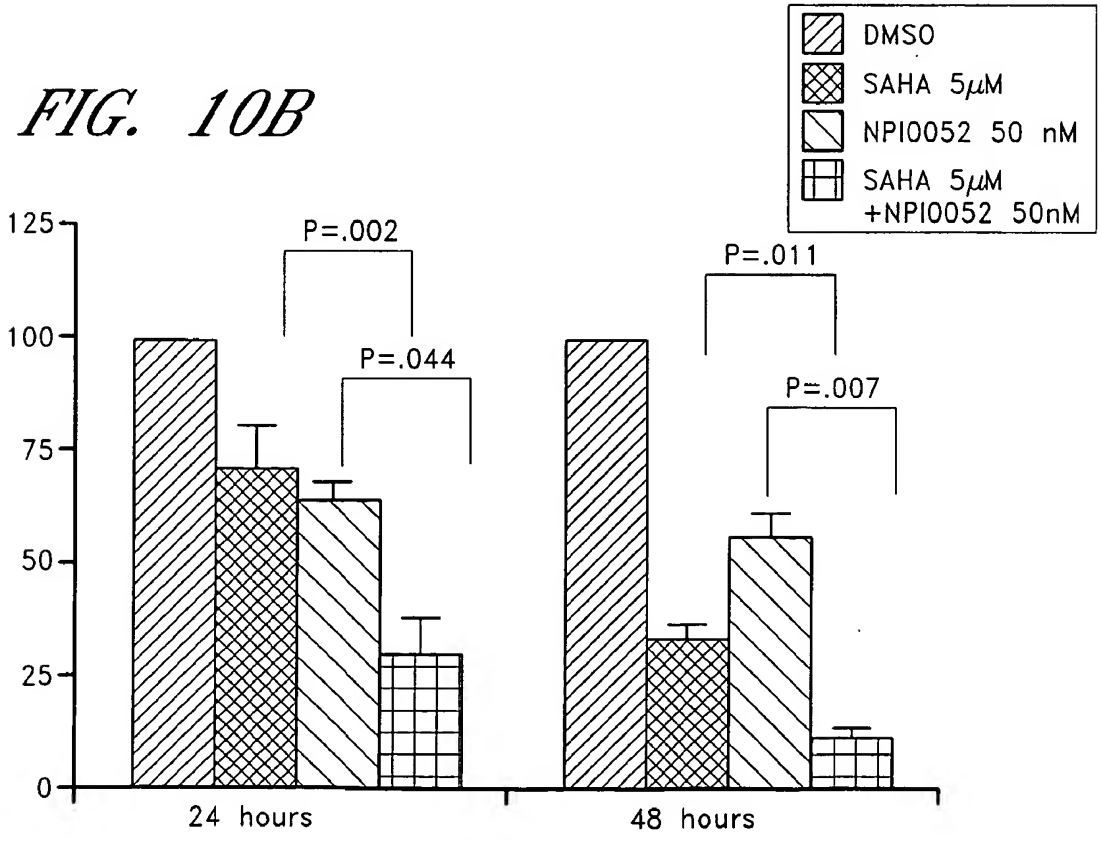


FIG. 10B



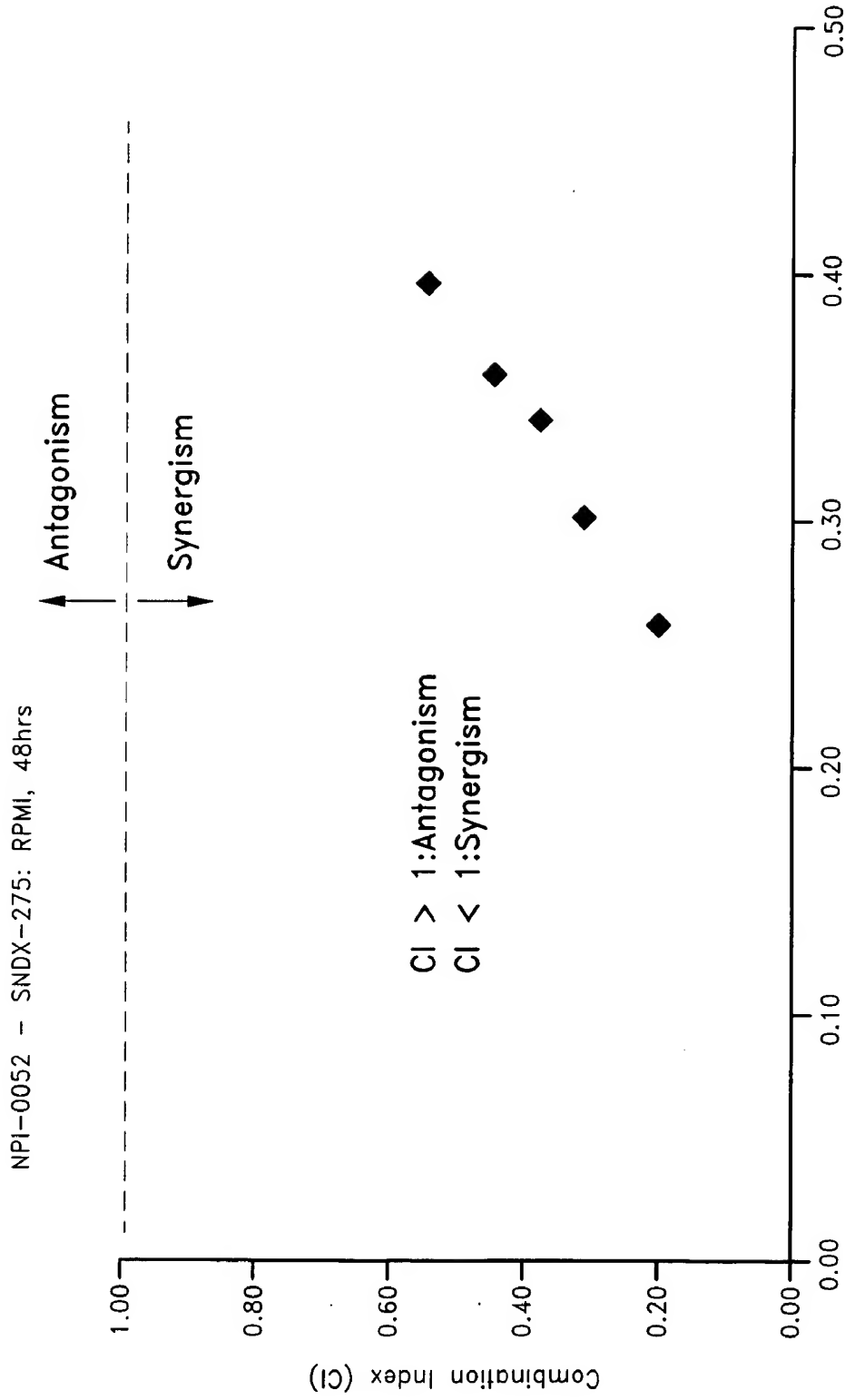

NPI-0052 - SNDX-275: RPMI, 48hrs
Antagonism
Synergism
CI > 1:Antagonism
CI < 1:Synergism
Combination Index (CI)
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.10
0.20
0.30
0.40
0.50
Fractional Effect

FIG. 11

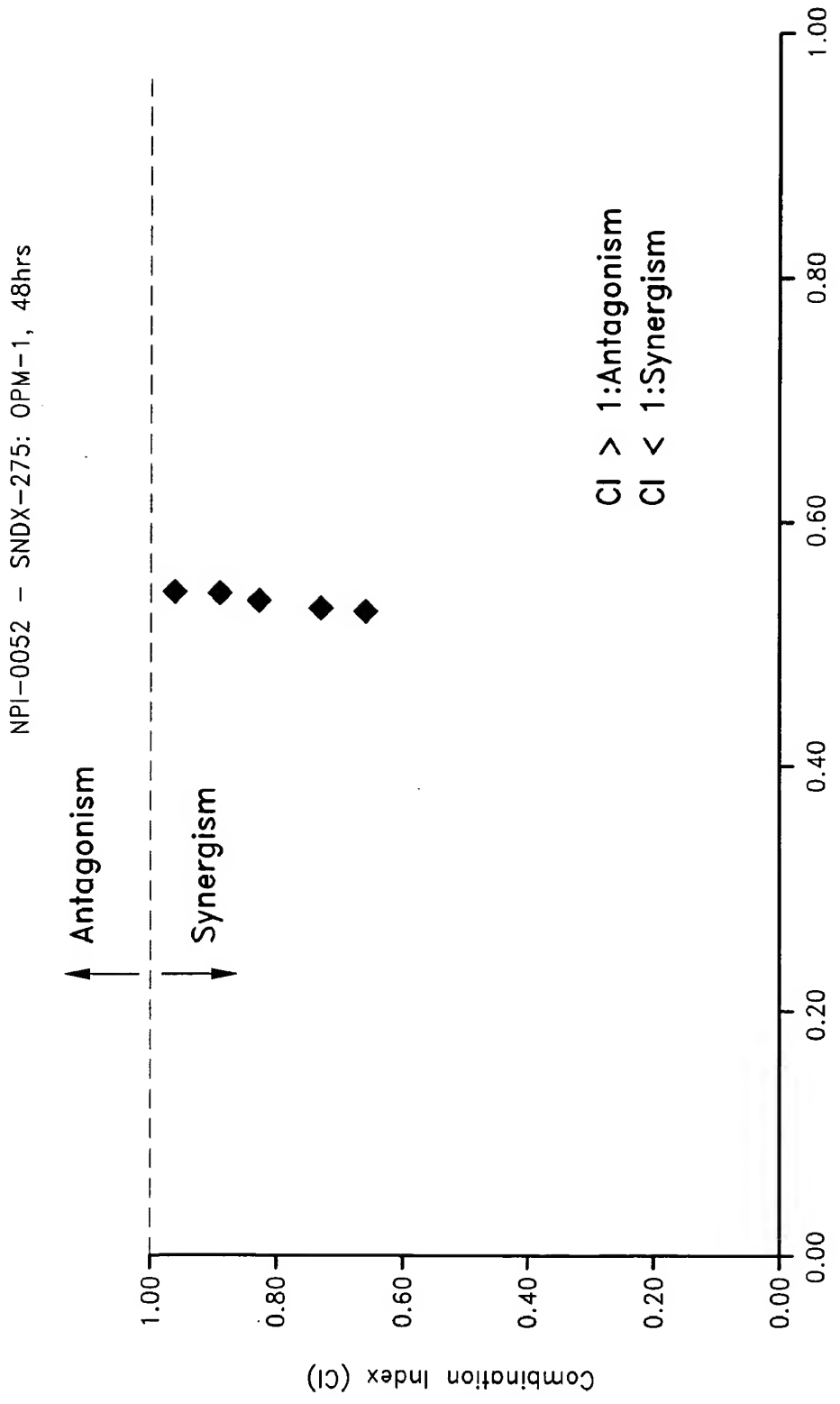

NPI-0052 - SNDX-275: OPM-1, 48hrs
Antagonism
Synergism
CI > 1:Antagonism
CI < 1:Synergism
Combination Index (CI)
Fractional Effect
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.20
0.40
0.60
0.80
1.00

FIG. 12

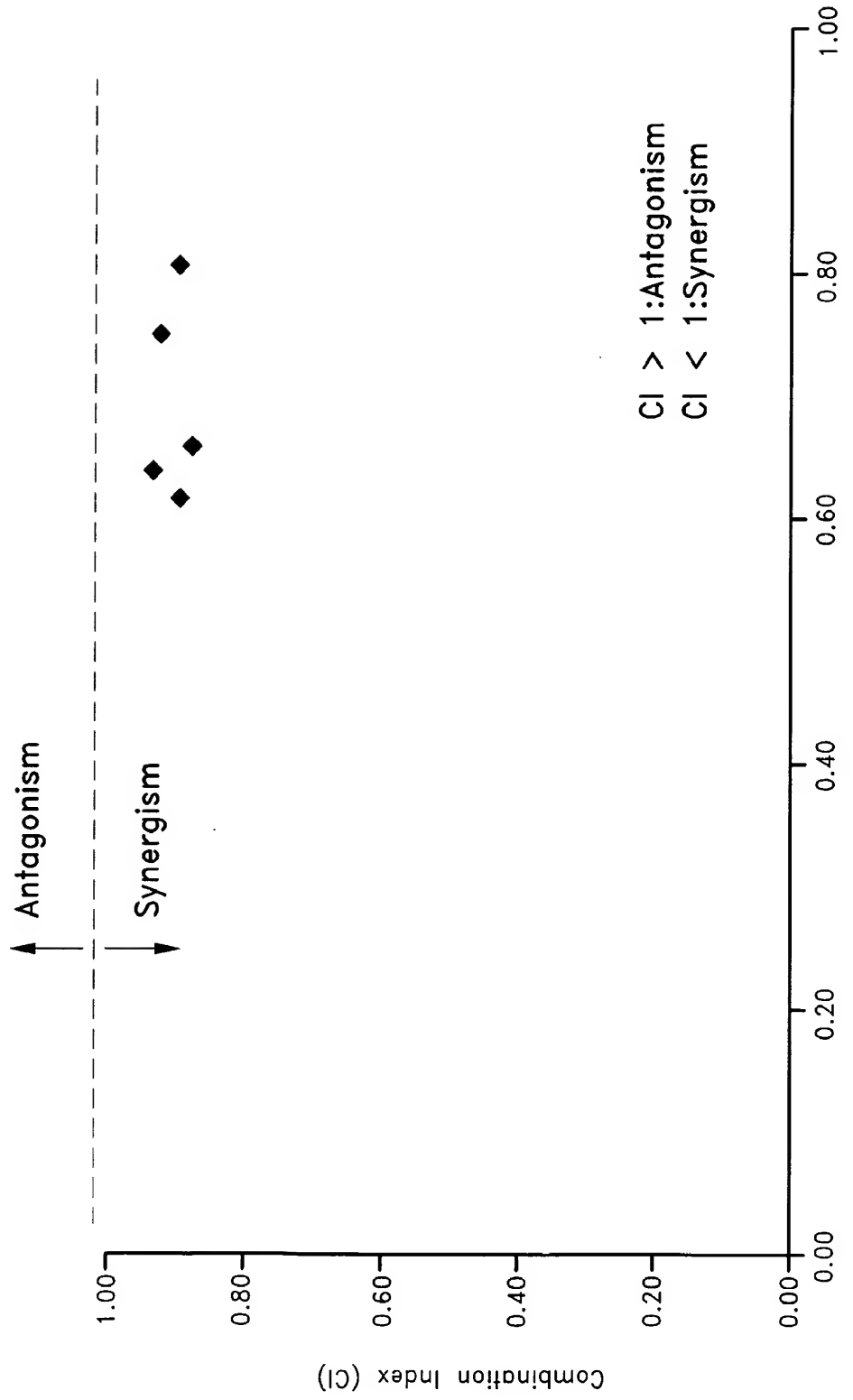

NPI-0052 - SNDX-275: DHL-6, 48hrs
Antagonism
Synergism
CI > 1:Antagonism
CI < 1:Synergism
Combination Index (CI)
Fractional Effect
0.00
0.20
0.40
0.60
0.80
1.00

FIG. 13

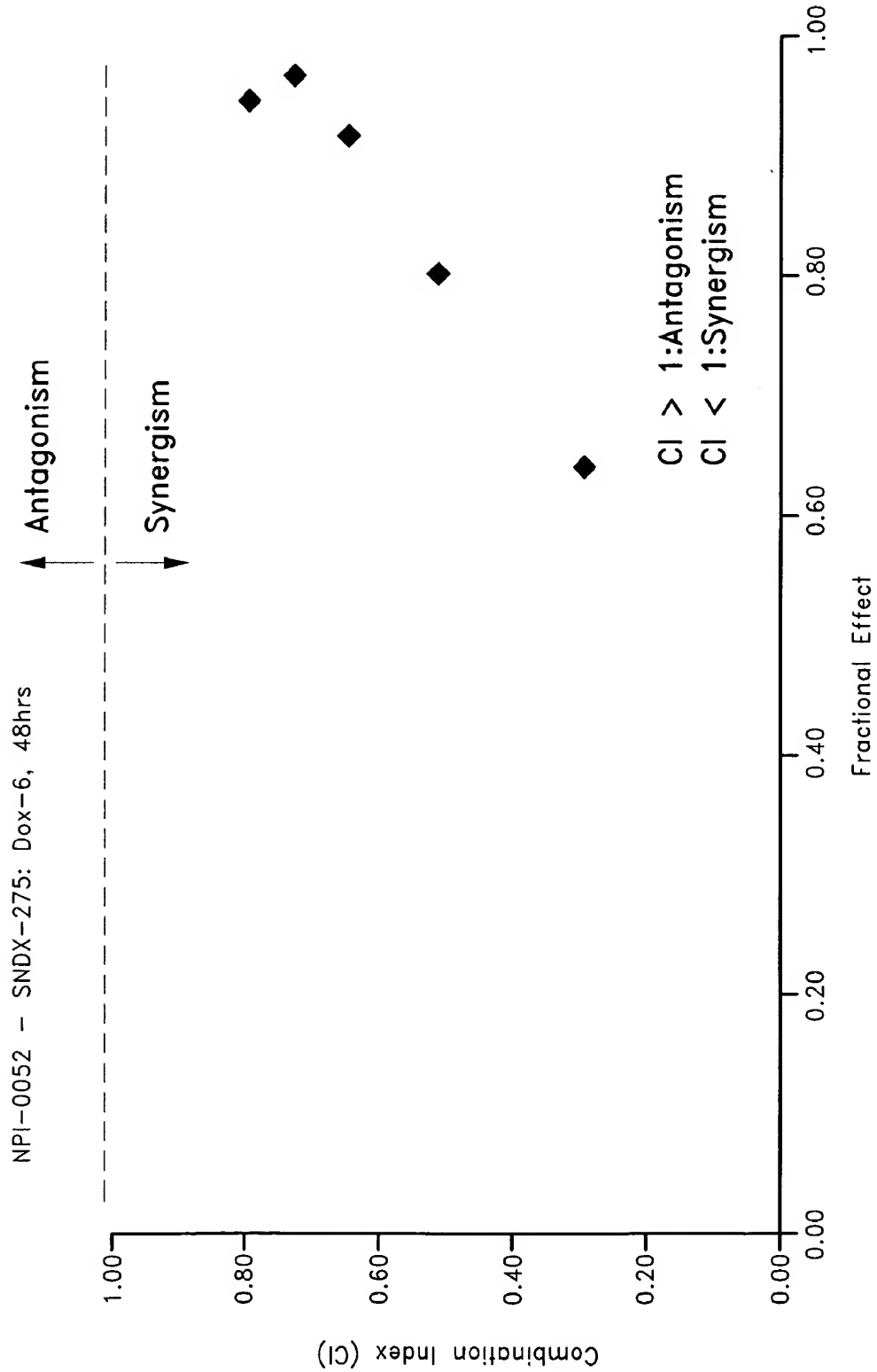

NPI-0052 - SNDX-275: Dox-6, 48hrs
Antagonism
Synergism
CI > 1:Antagonism
CI < 1:Synergism
Combination Index (CI)
Fractional Effect
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.20
0.40
0.60
0.80
1.00

FIG. 14

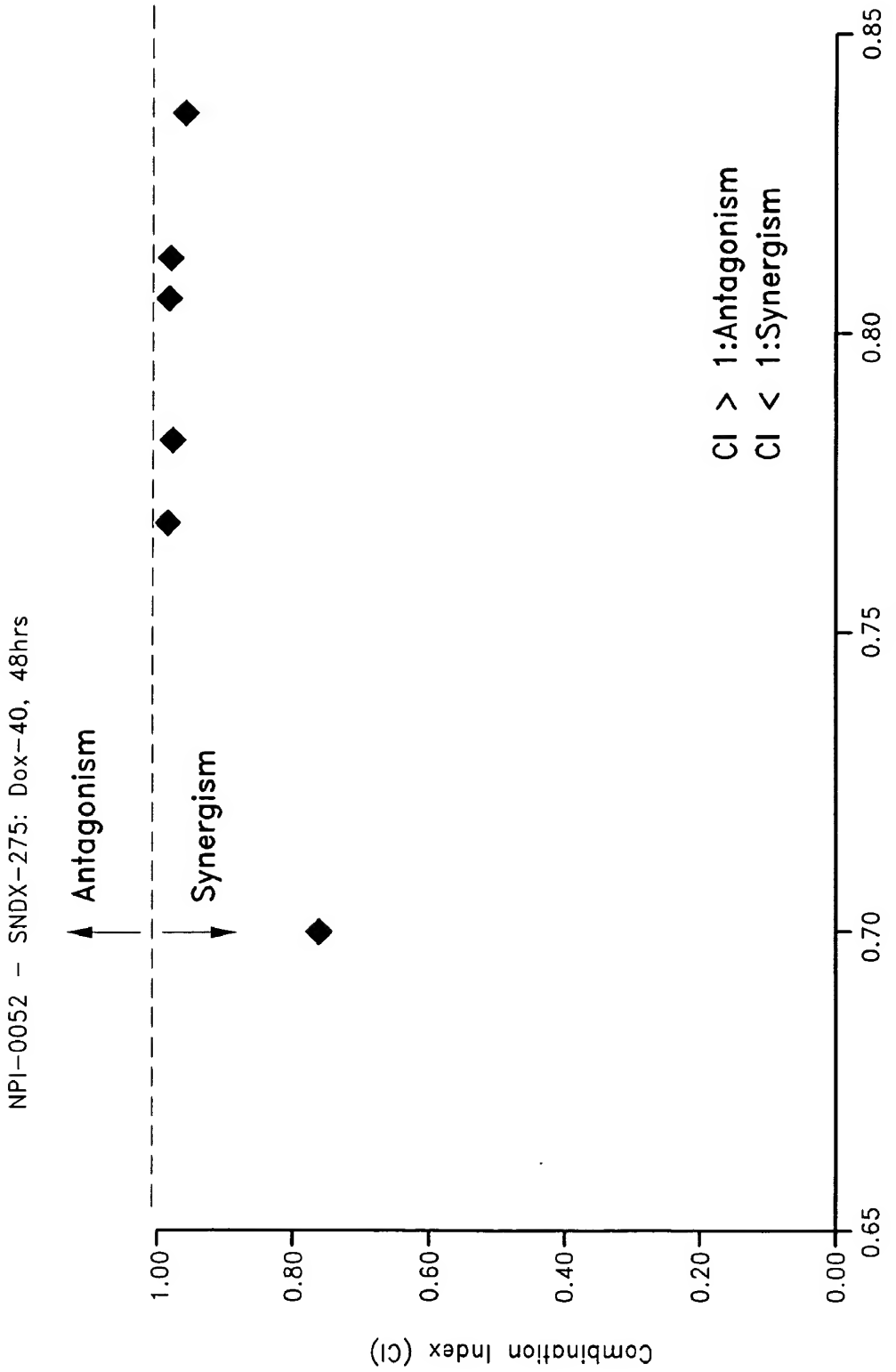

NPI-0052 - SNDX-275: Dox-40, 48hrs
Antagonism
Synergism
CI > 1:Antagonism
CI < 1:Synergism
Combination Index (CI)
Fractional Effect
1.00
0.80
0.60
0.40
0.20
0.00
0.65
0.70
0.75
0.80
0.85

FIG. 15

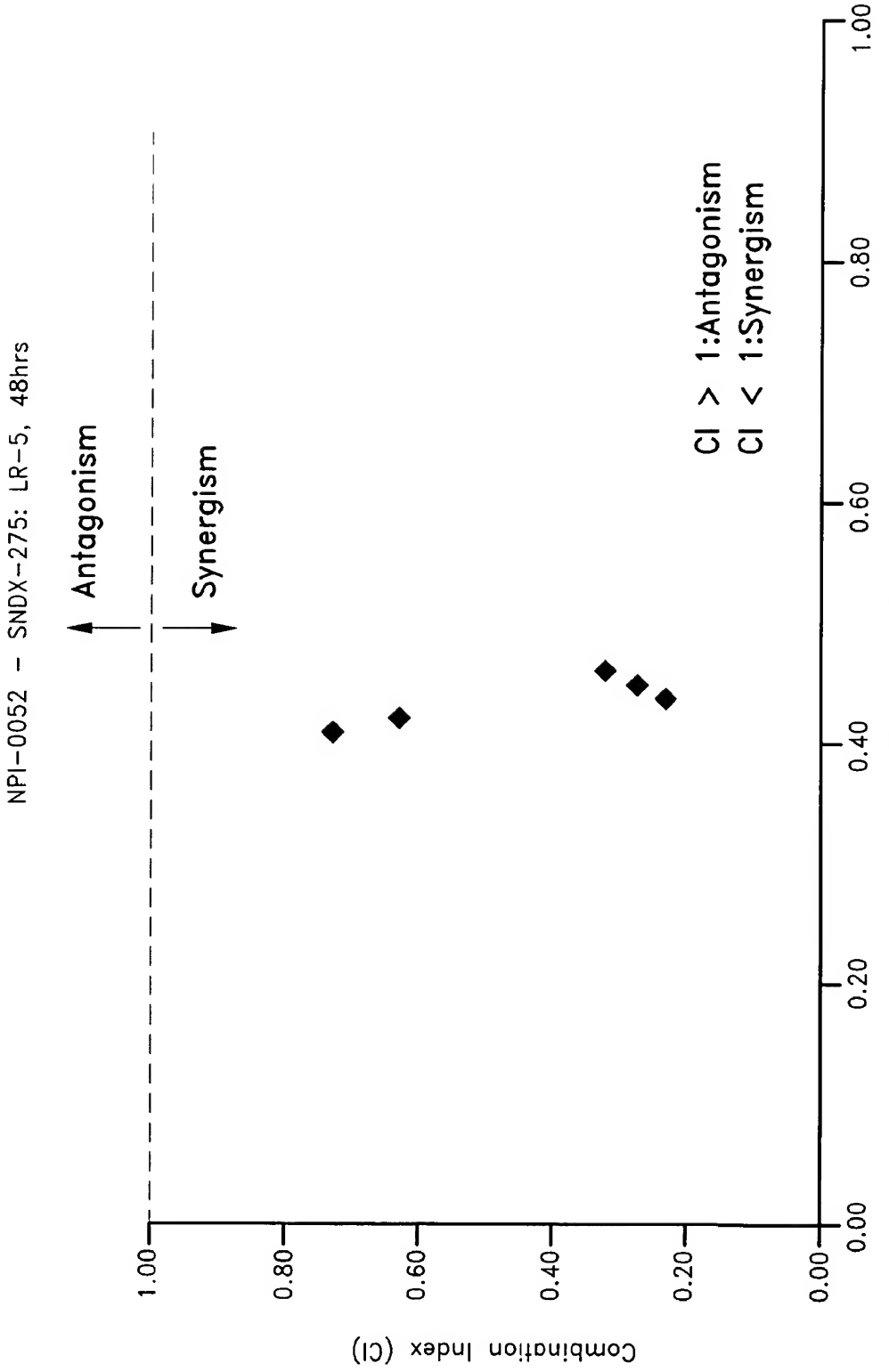

NPI-0052 - SNDX-275: LR-5, 48hrs
Antagonism
Synergism
CI > 1:Antagonism
CI < 1:Synergism
Combination Index (CI)
Fractional Effect
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.20
0.40
0.60
0.80
1.00

FIG. 16

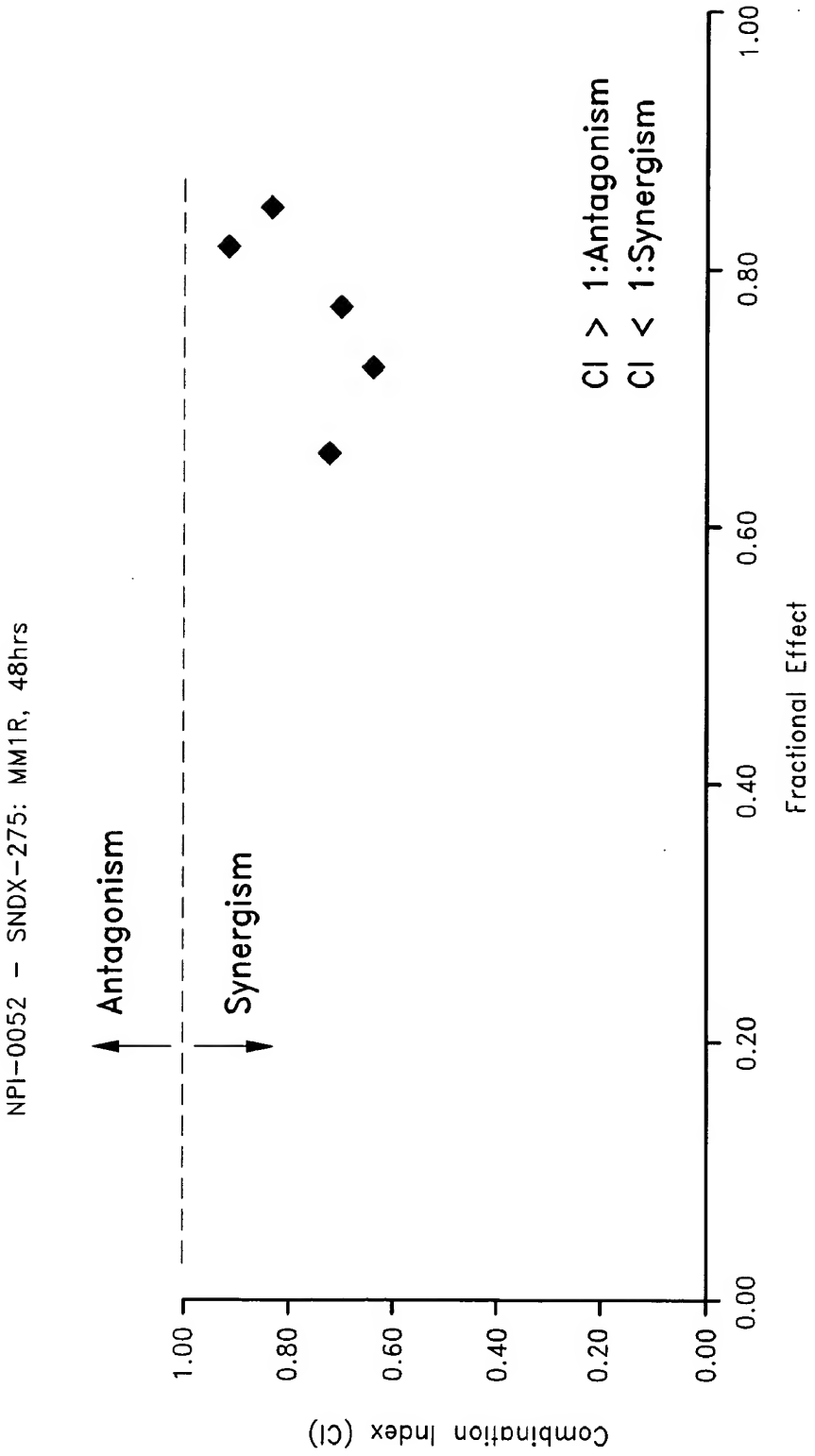

NPI-0052 - SNDX-275: MM1R, 48hrs
Antagonism
Synergism
CI > 1:Antagonism
CI < 1:Synergism
Combination Index (CI)
Fractional Effect
1.00
0.80
0.60
0.40
0.20
0.00
0.00
0.20
0.40
0.60
0.80
1.00

FIG. 17

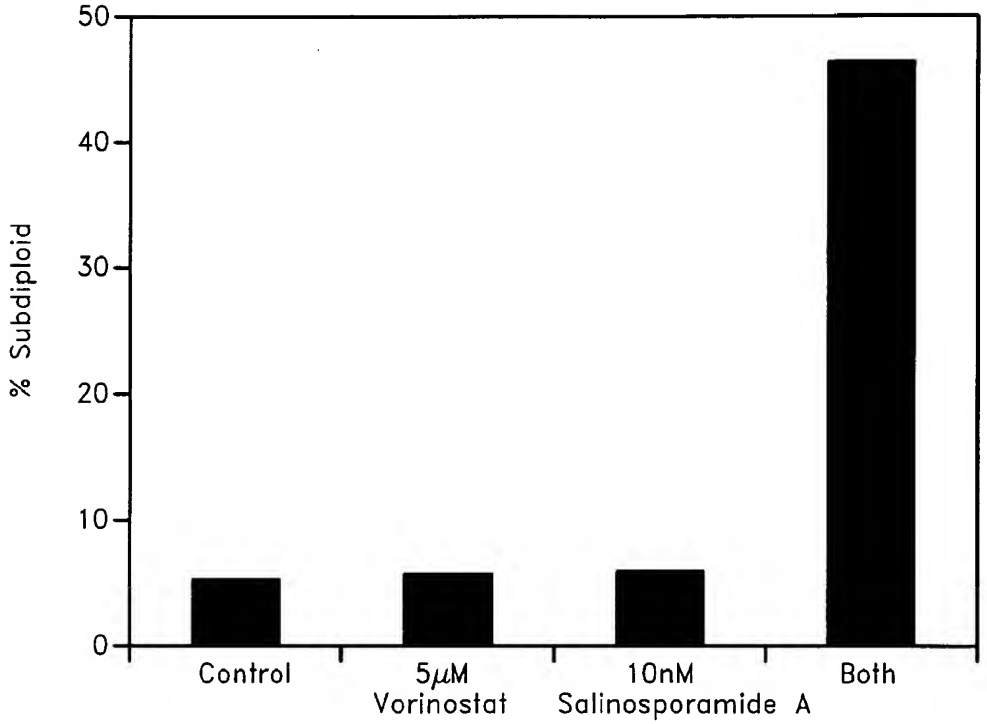


FIG. 18A

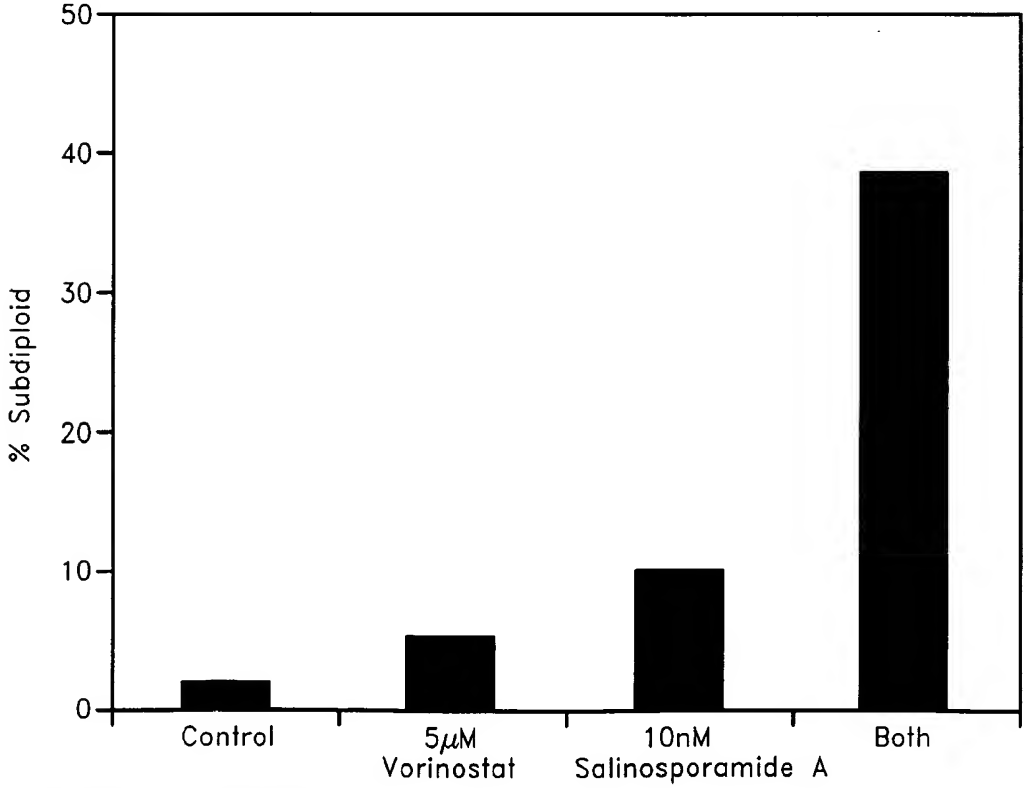


FIG. 18B

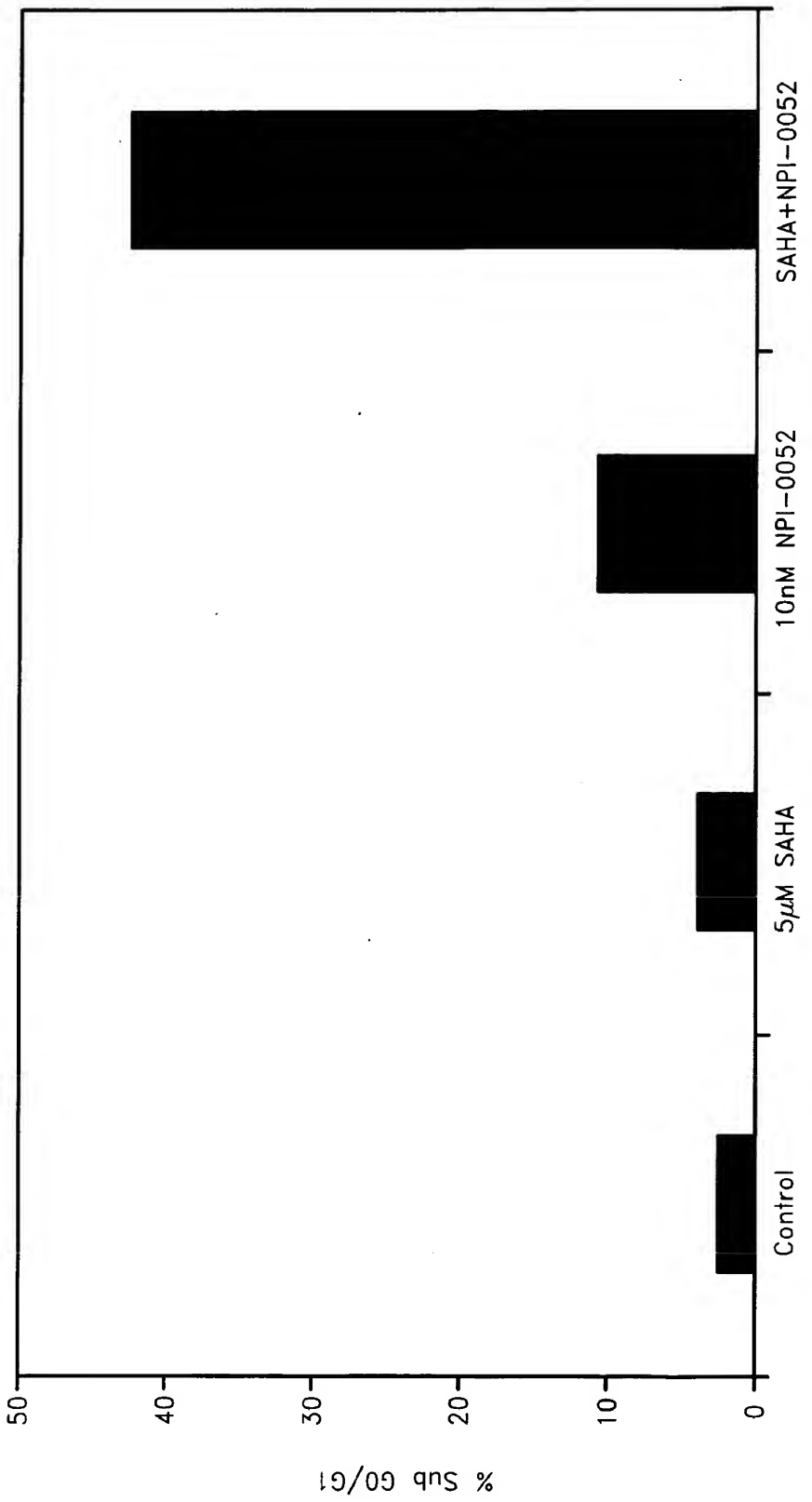

% Sub G0/G1
0
10
20
30
40
50
Control
5μM SAHA
10nM NPI-0052
SAHA+NPI-0052

FIG. 19

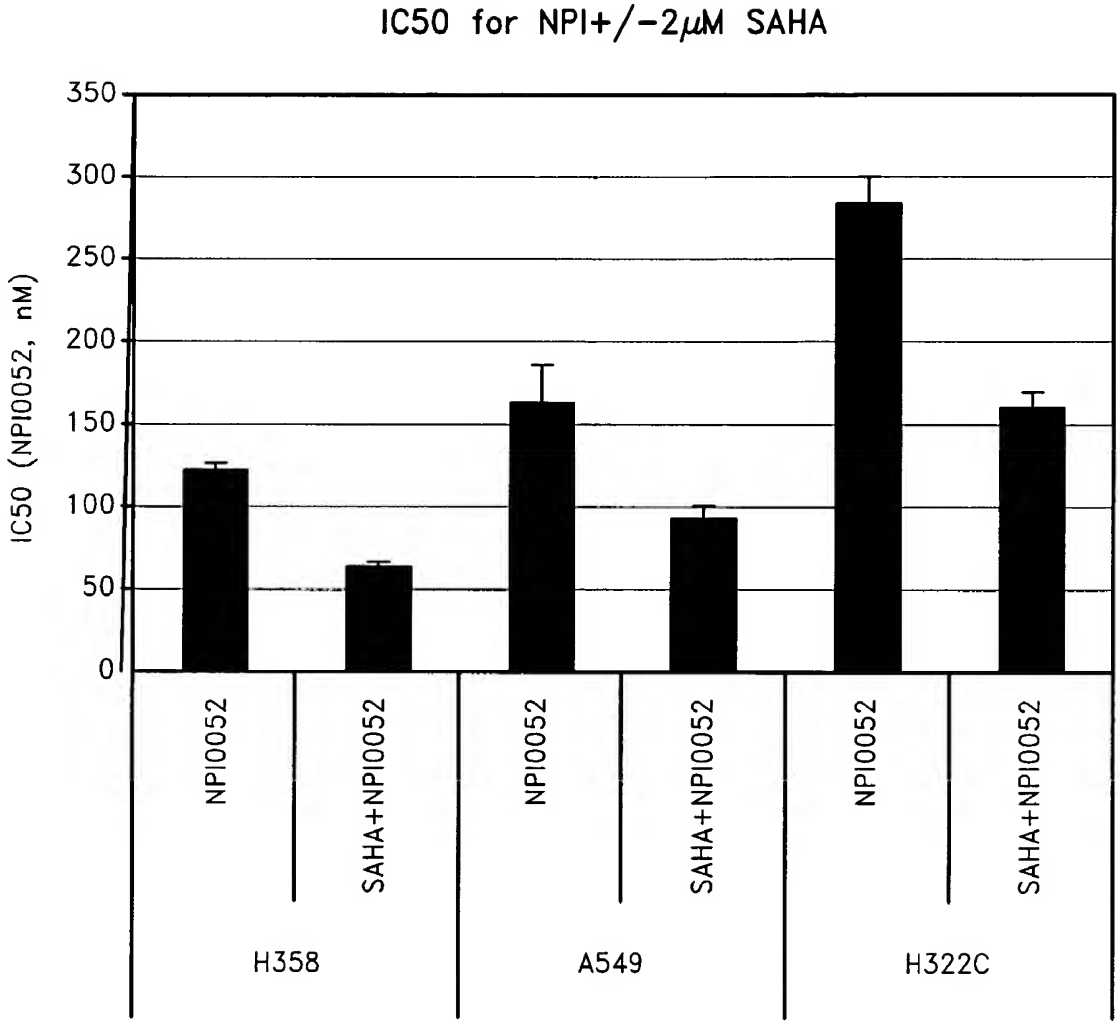

IC50 for NPI+/−2μM SAHA
IC50 (NPI0052, nM)
350
300
250
200
150
100
50
0
NPI0052
SAHA+NPI0052
NPI0052
SAHA+NPI0052
NPI0052
SAHA+NPI0052
H358
A549
H322C

FIG. 20

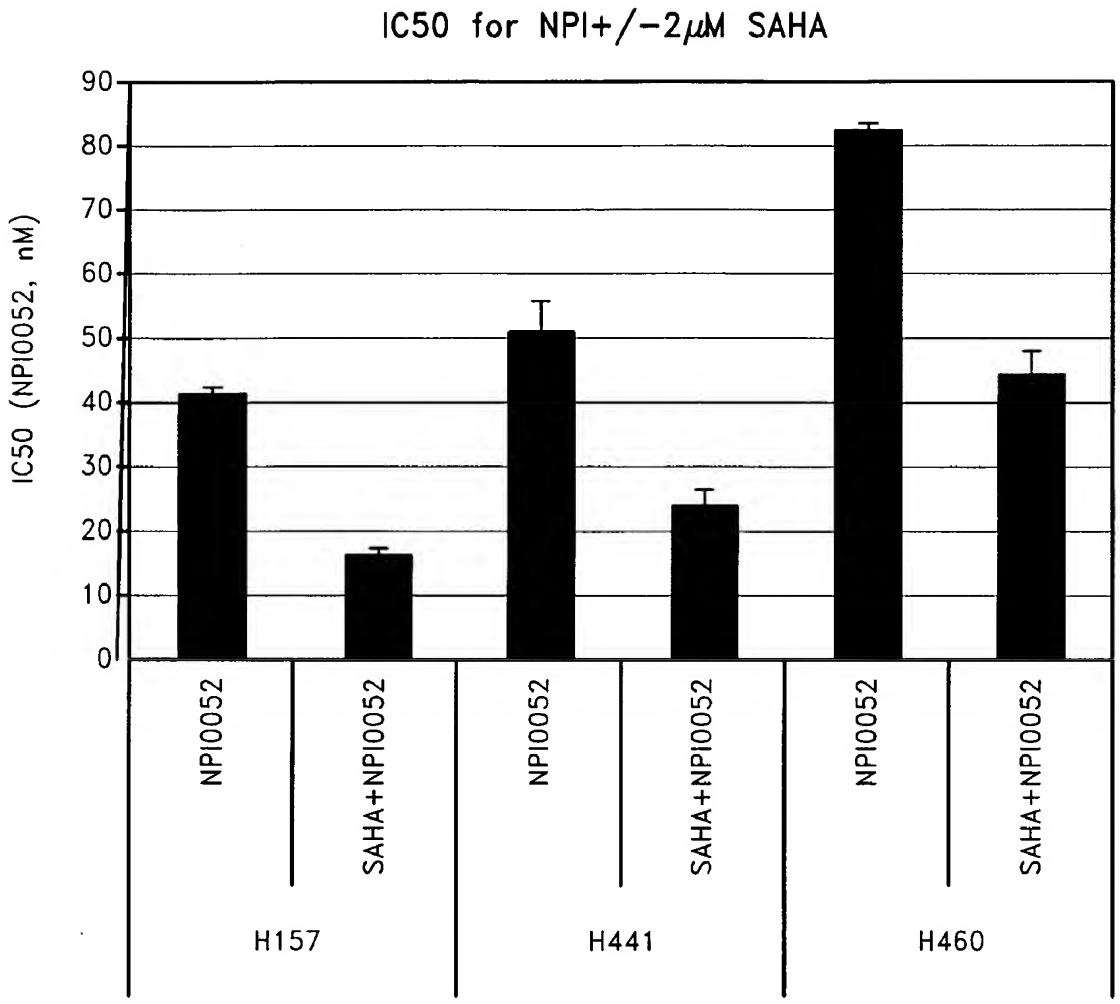

IC50 for NPI+/−2μM SAHA
IC50 (NPI0052, nM)
90
80
70
60
50
40
30
20
10
0
NPI0052
SAHA+NPI0052
NPI0052
SAHA+NPI0052
NPI0052
SAHA+NPI0052
H157
H441
H460

FIG. 21

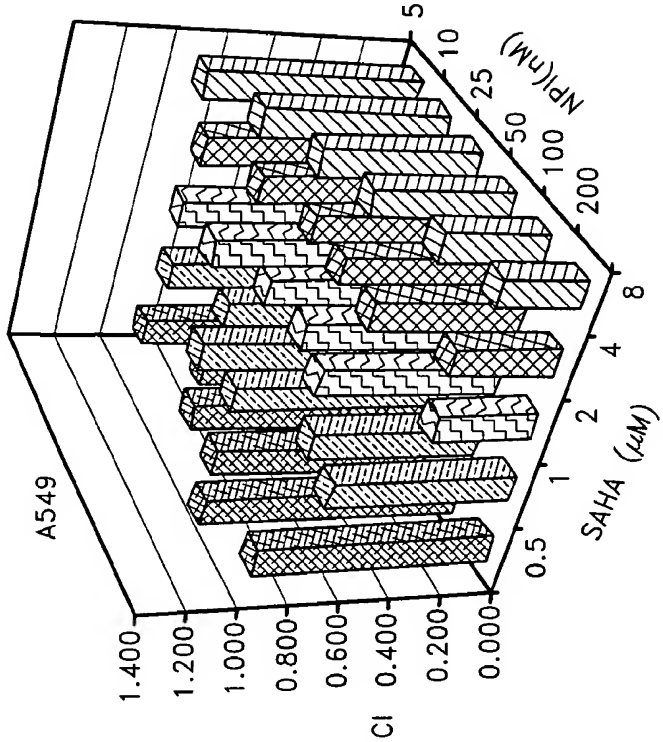


FIG. 22B

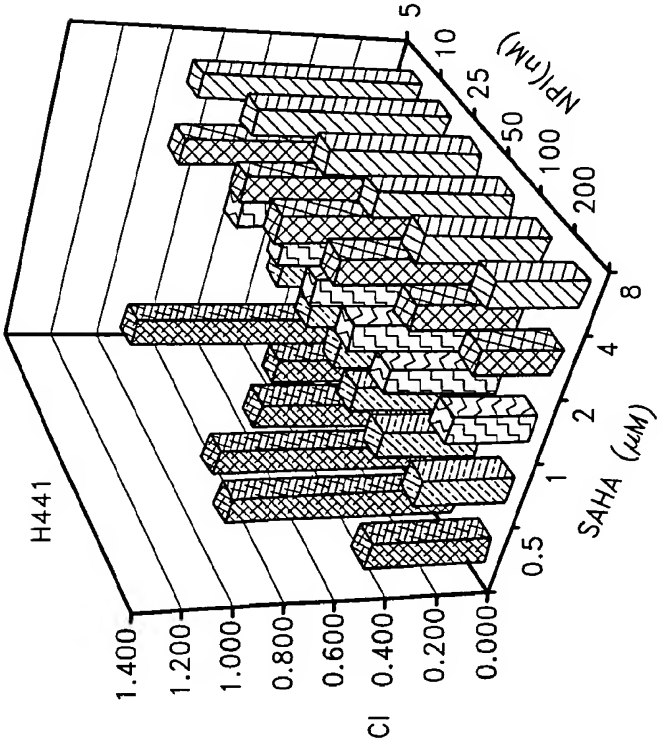


FIG. 22A

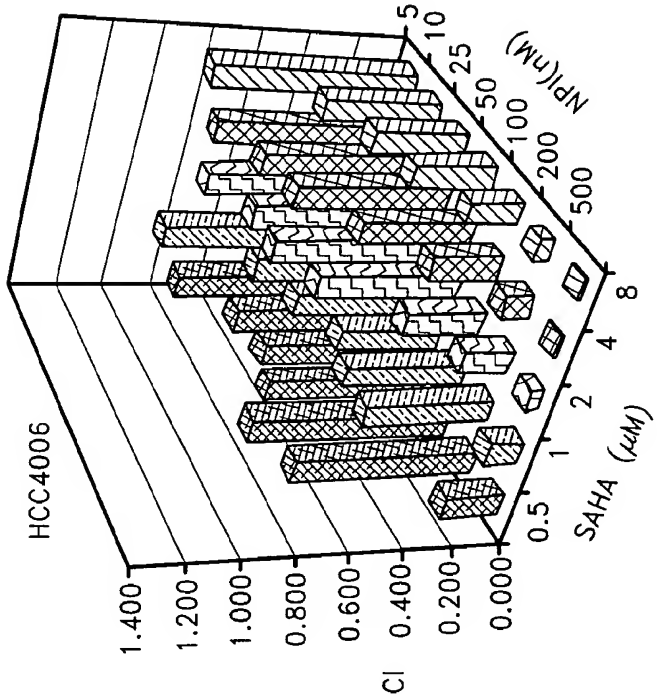


FIG. 22D

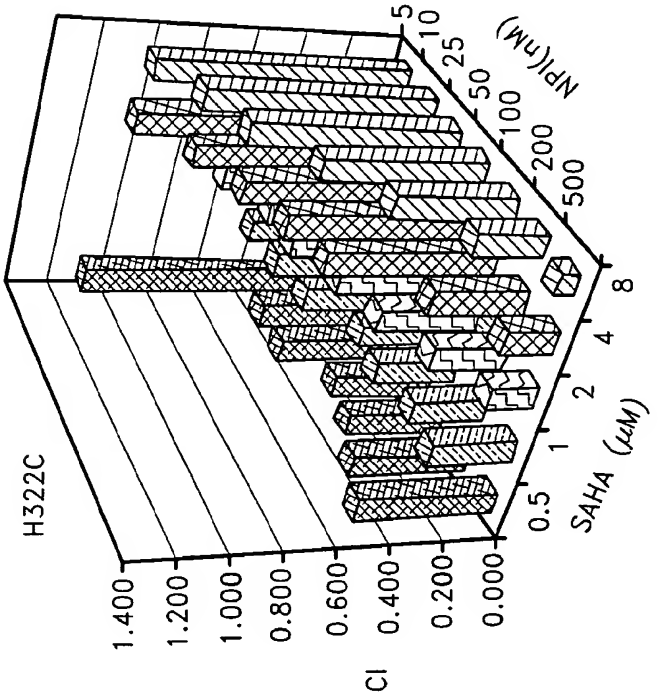


FIG. 22C

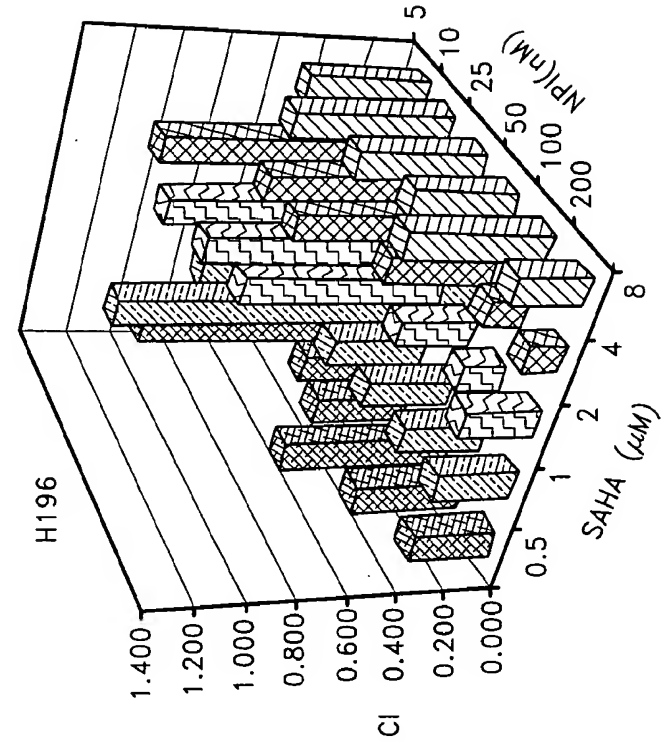


FIG. 22F

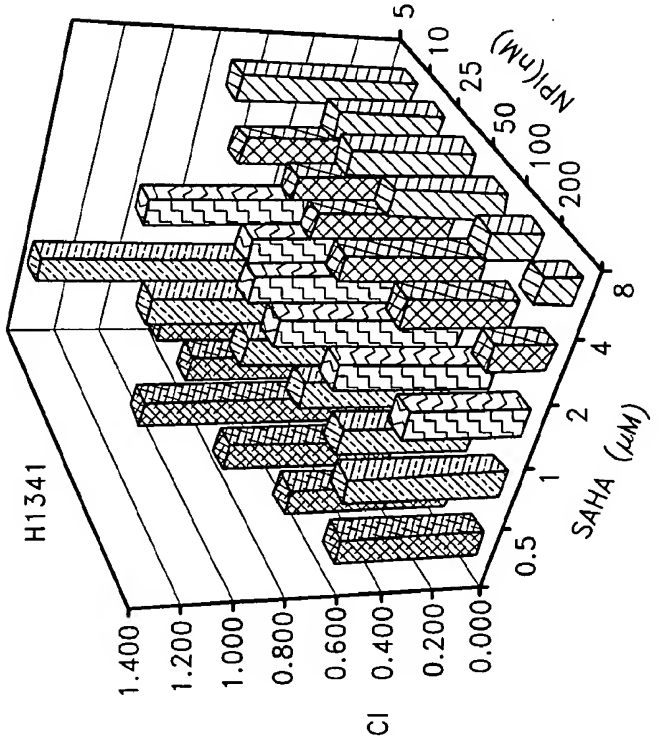


FIG. 22E

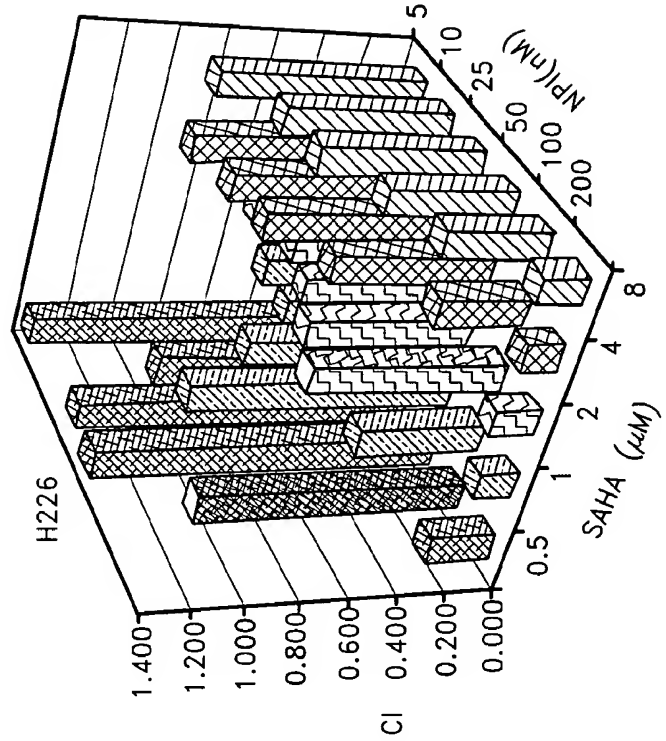


FIG. 22H

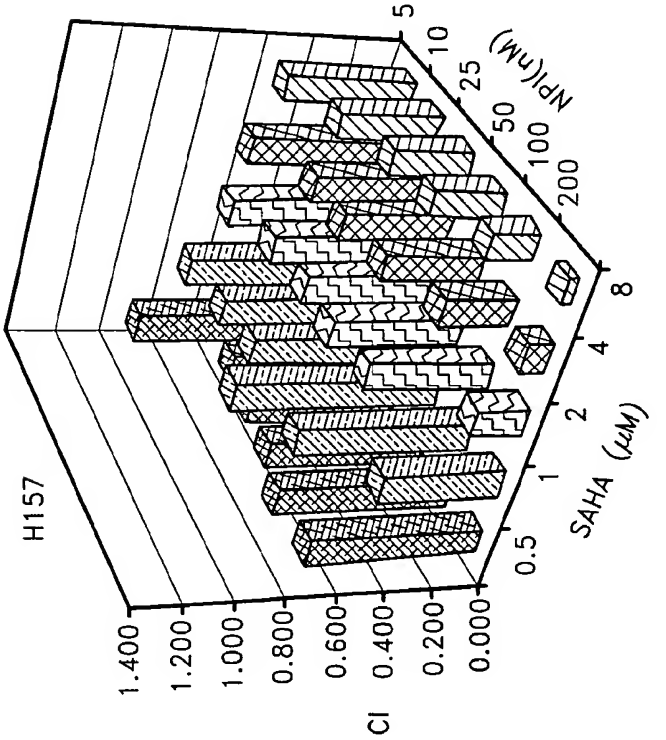


FIG. 22G

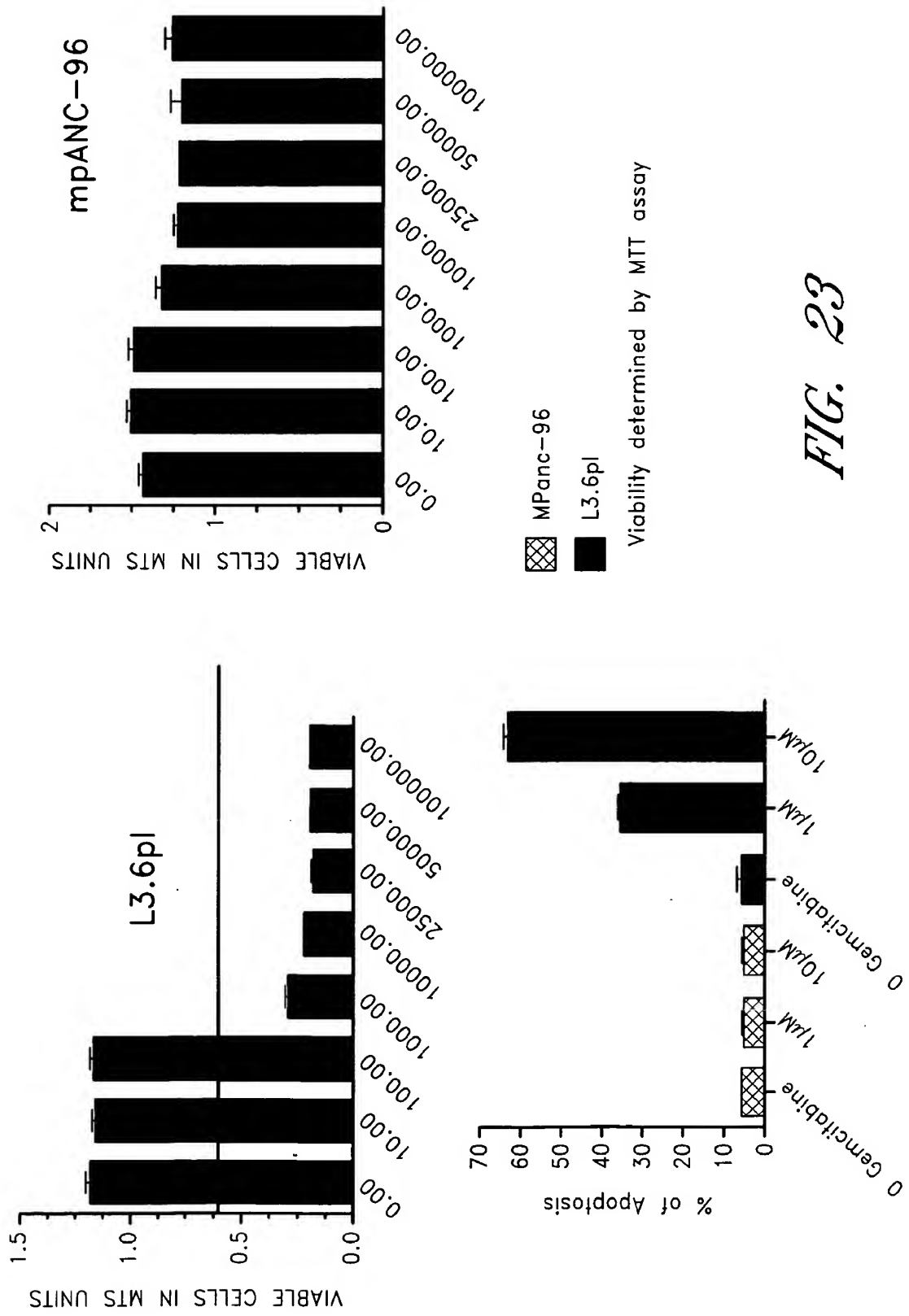
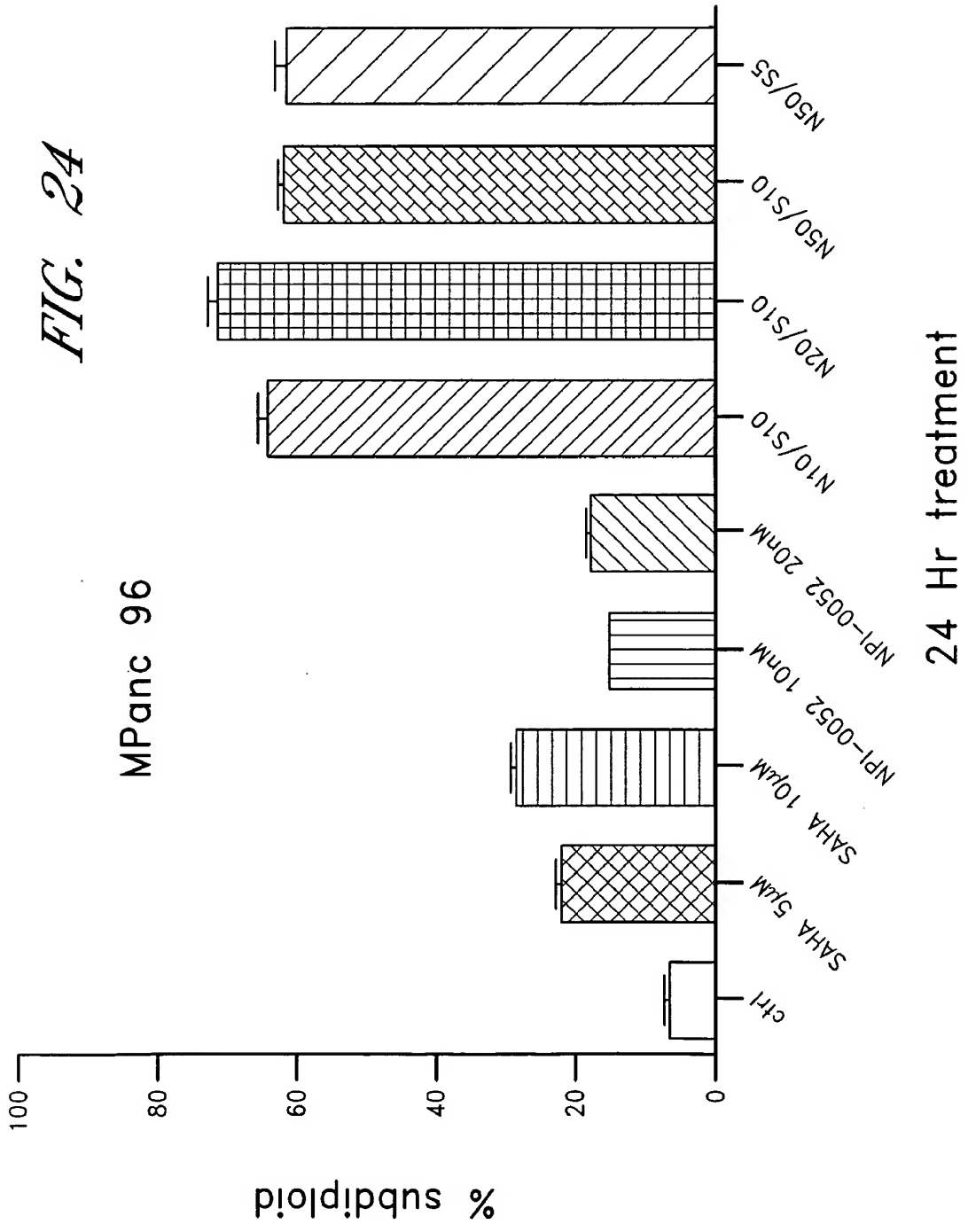

mpANC-96
VIABLE CELLS IN MTS UNITS
0
1
2
0.00
10.00
100.00
1000.00
10000.00
25000.00
50000.00
100000.00
MPanc-96
L3.6pl
Viability determined by MTT assay
L3.6pl
VIABLE CELLS IN MTS UNITS
0.0
0.5
1.0
1.5
% of Apoptosis
0
10
20
30
40
50
60
70
0 Gemcitabine
1μM
10μM
0 Gemcitabine
1μM
10μM

FIG. 23

FIG. 24


MPanc 96
% subdiploid
100
80
60
40
20
0
ctrl
SAHA 5μM
SAHA 10μM
NPI-0052 10nM
NPI-0052 20nM
N10/S10
N20/S10
N50/S10
N50/S5
24 Hr treatment

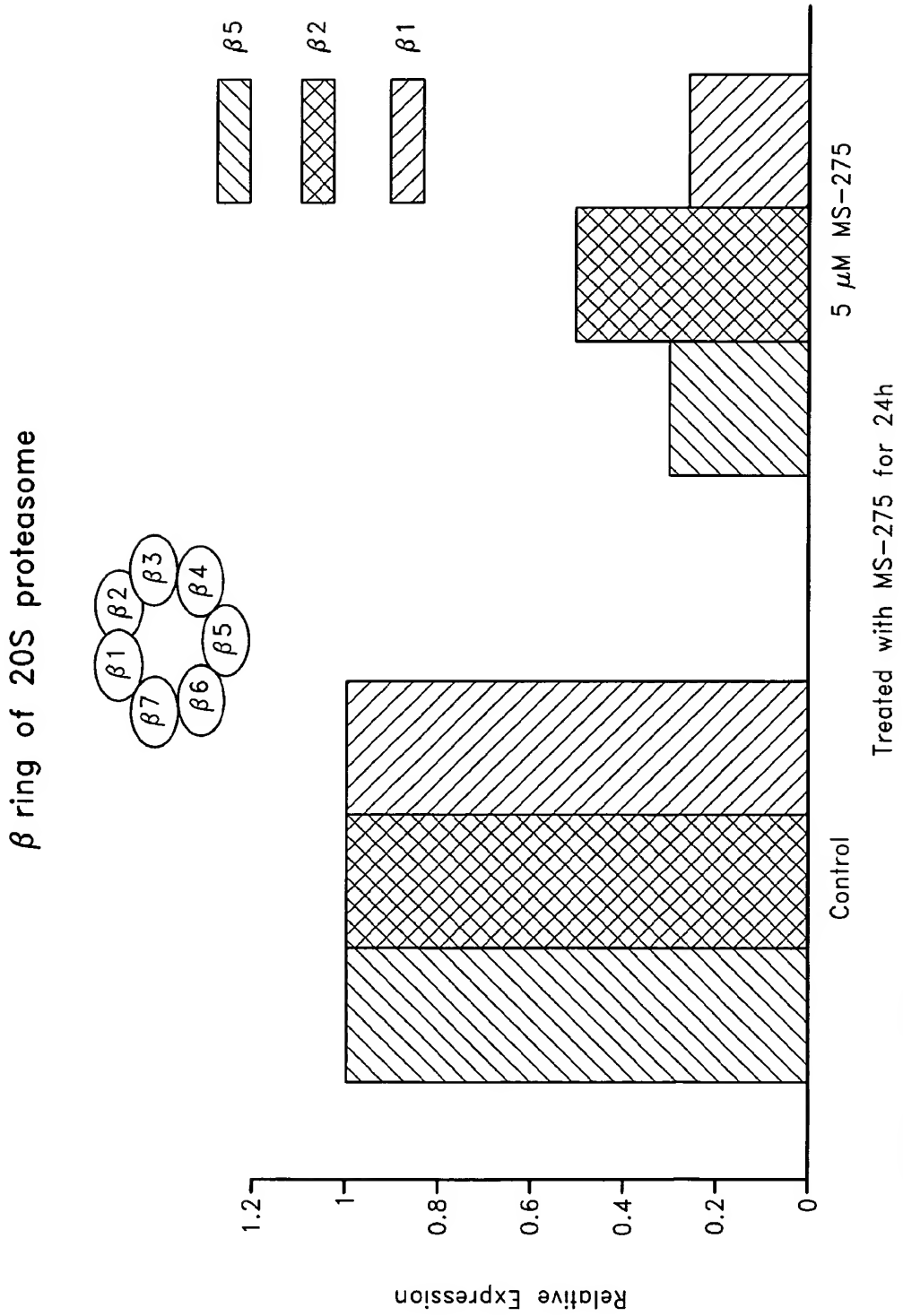

β ring of 20S proteasome
β1
β2
β3
β4
β5
β6
β7
β5
β2
β1
Relative Expression
1.2
1
0.8
0.6
0.4
0.2
0
Control
Treated with MS-275 for 24h
5 μM MS-275

FIG. 25

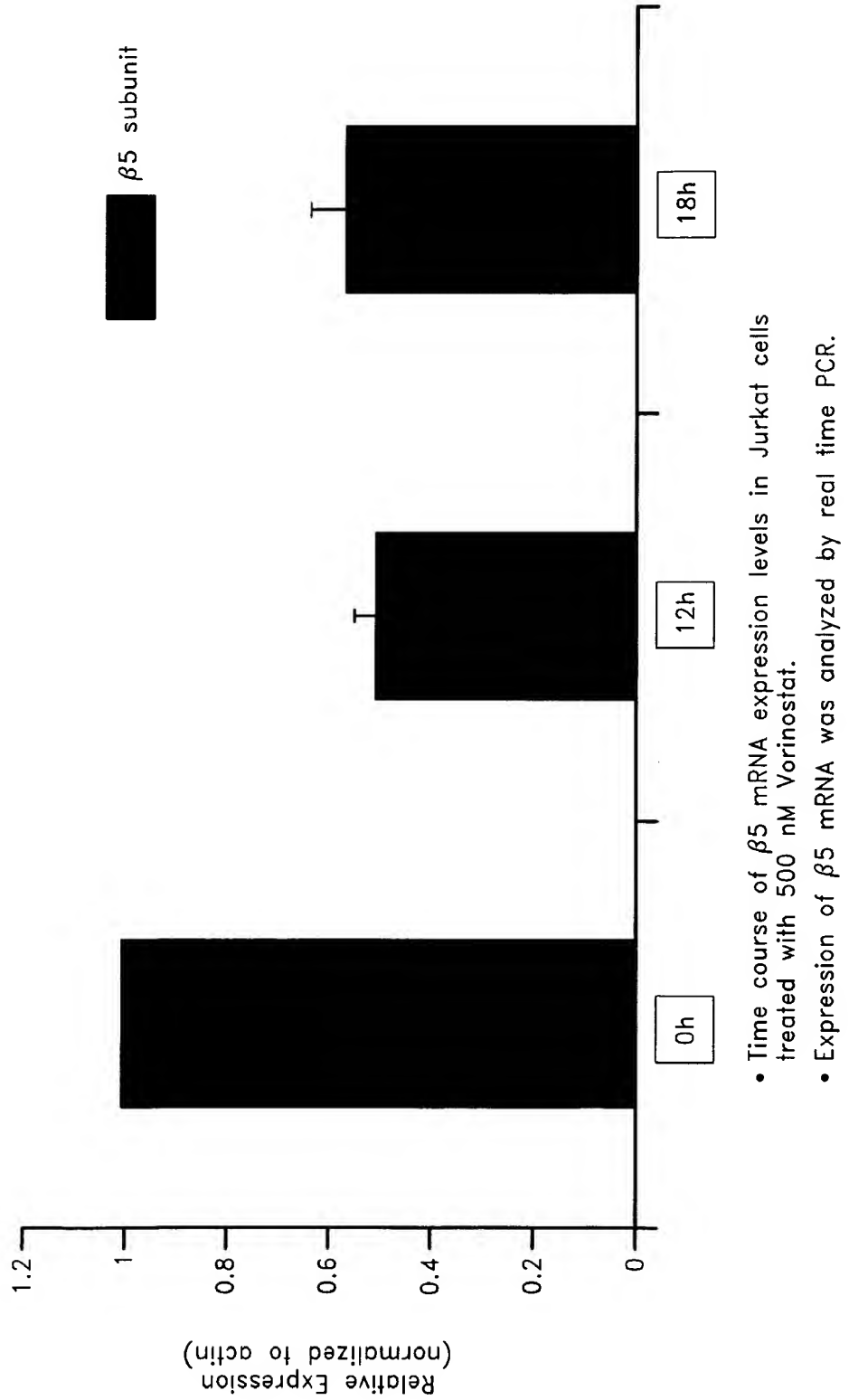

β5 subunit
Relative Expression
(normalized to actin)
1.2
1
0.8
0.6
0.4
0.2
0
0h
12h
18h
• Time course of β5 mRNA expression levels in Jurkat cells treated with 500 nM Vorinostat.
• Expression of β5 mRNA was analyzed by real time PCR.

FIG. 26

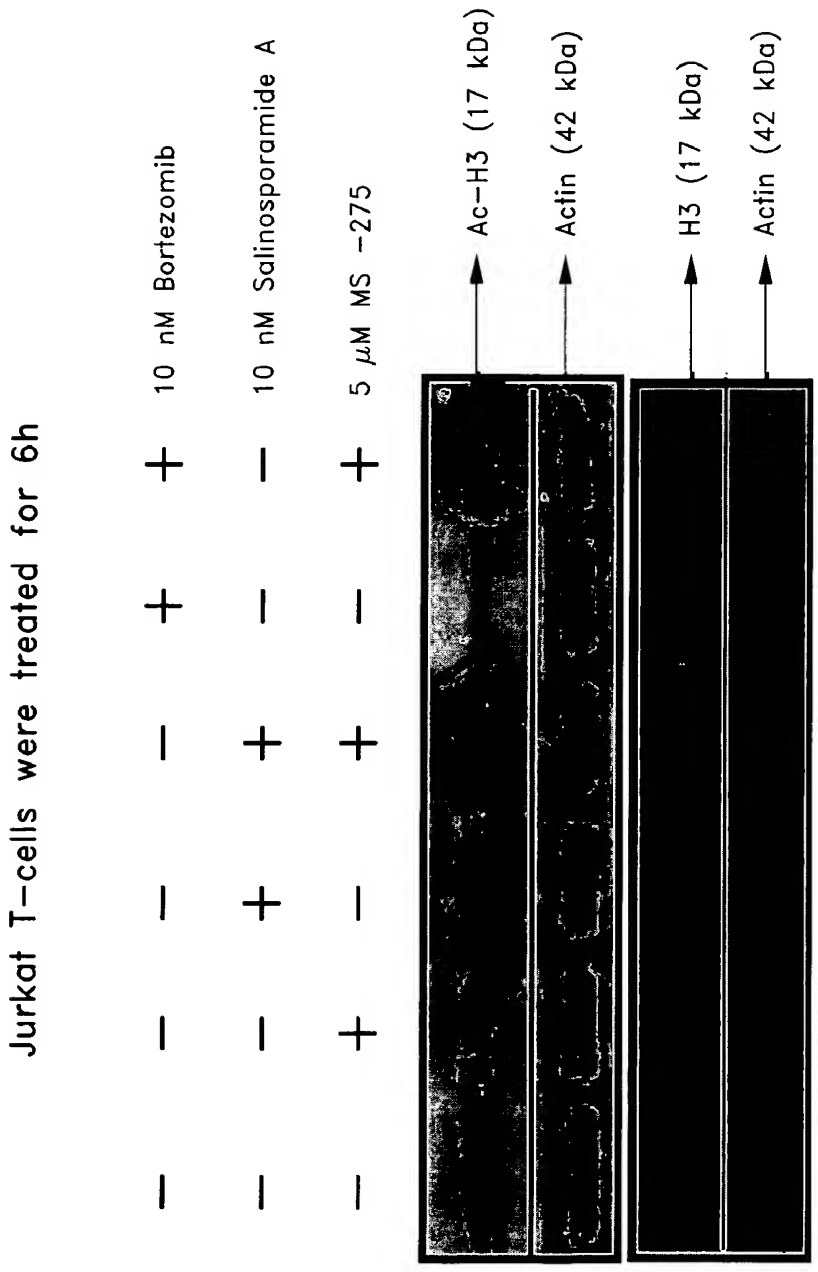
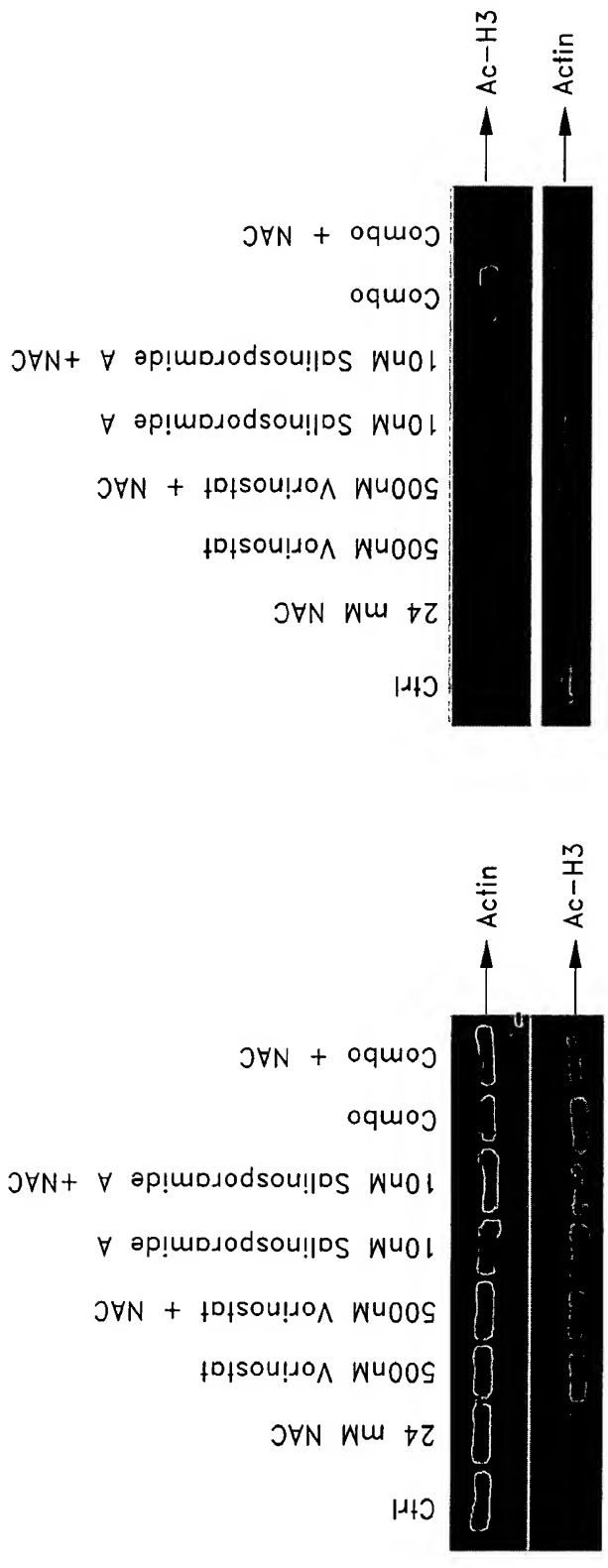

Jurkat T-cells were treated for 6h
10 nM Bortezomib
10 nM Salinosporamide A
5 μM MS -275
Ac-H3 (17 kDa)
Actin (42 kDa)
H3 (17 kDa)
Actin (42 kDa)

FIG. 27



Cells were treated for 6h as indicated

FIG. 28

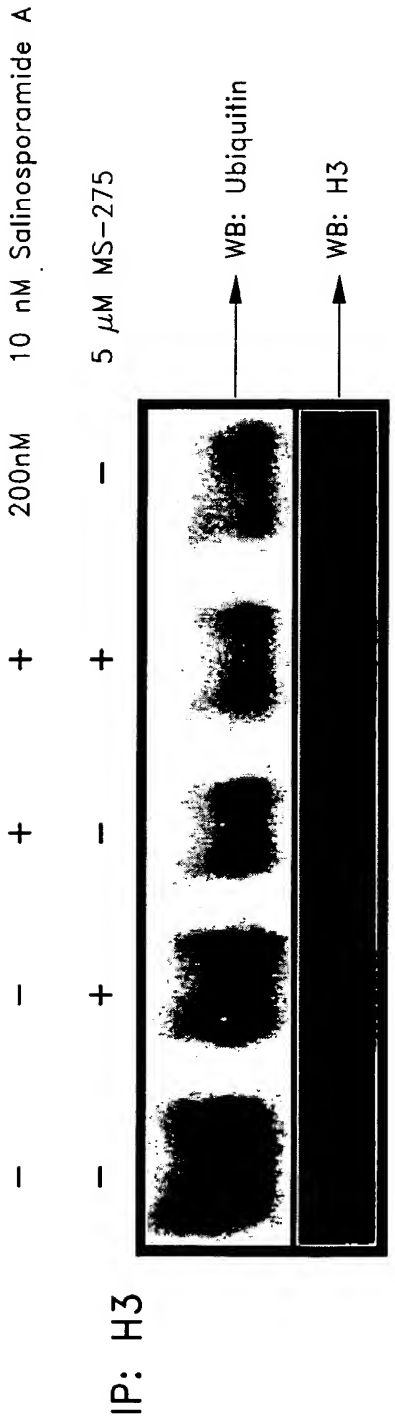

10 nM Salinosporamide A
200nM
5 μM MS-275
WB: Ubiquitin
WB: H3
IP: H3

FIG. 29

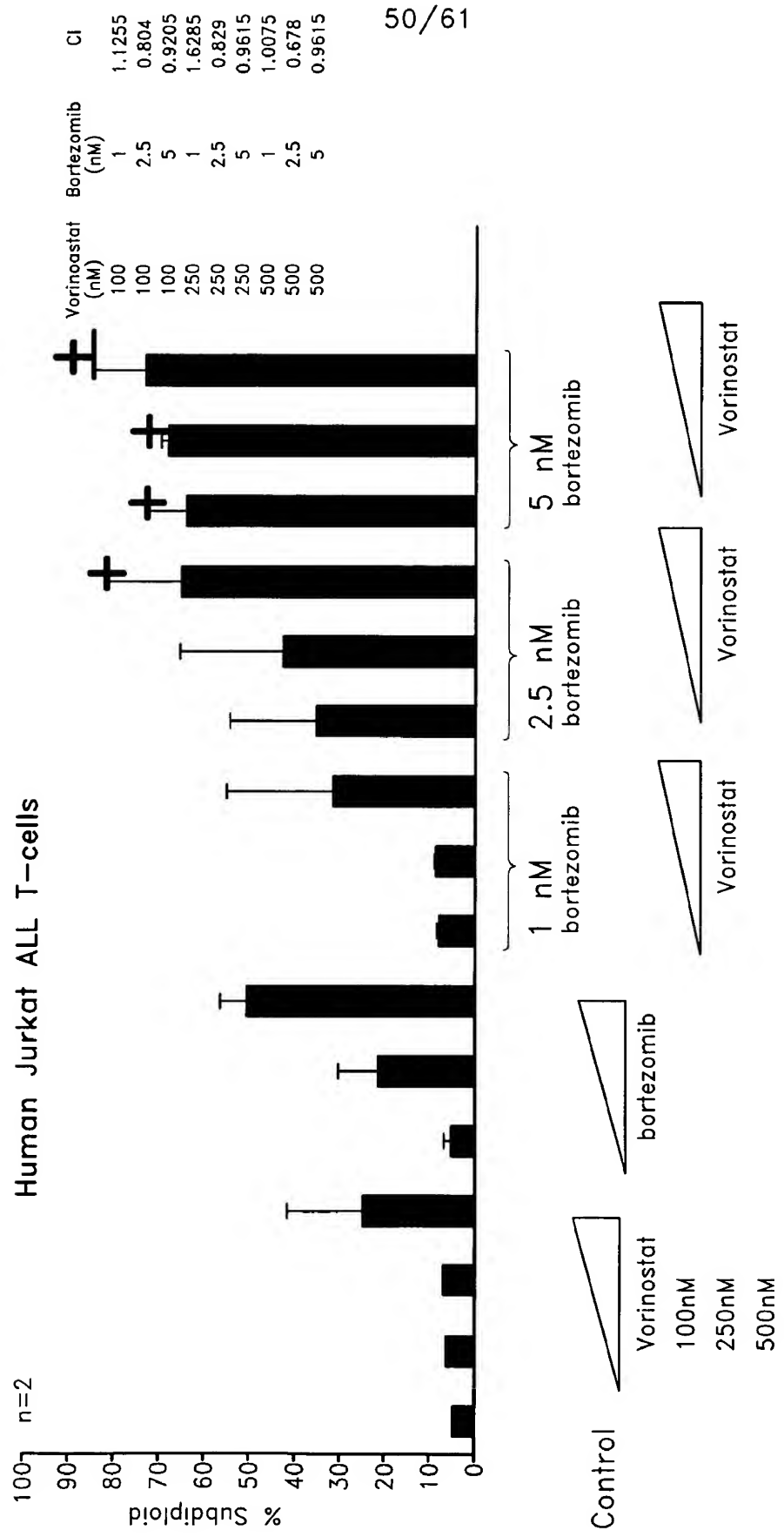

Human Jurkat ALL T-cells
n=2
% Subdiploid
100
90
80
70
60
50
40
30
20
10
0
Vorinoastat (nM)
Bortezomib (nM)
CI
100 1 1.1255
100 2.5 0.804
100 5 0.9205
250 1 1.6285
250 2.5 0.829
250 5 0.9615
500 1 1.0075
500 2.5 0.678
500 5 0.9615
1 nM bortezomib
2.5 nM bortezomib
5 nM bortezomib
Control
Vorinostat 100nM 250nM 500nM
bortezomib
Vorinostat
Vorinostat
Vorinostat

FIG. 30

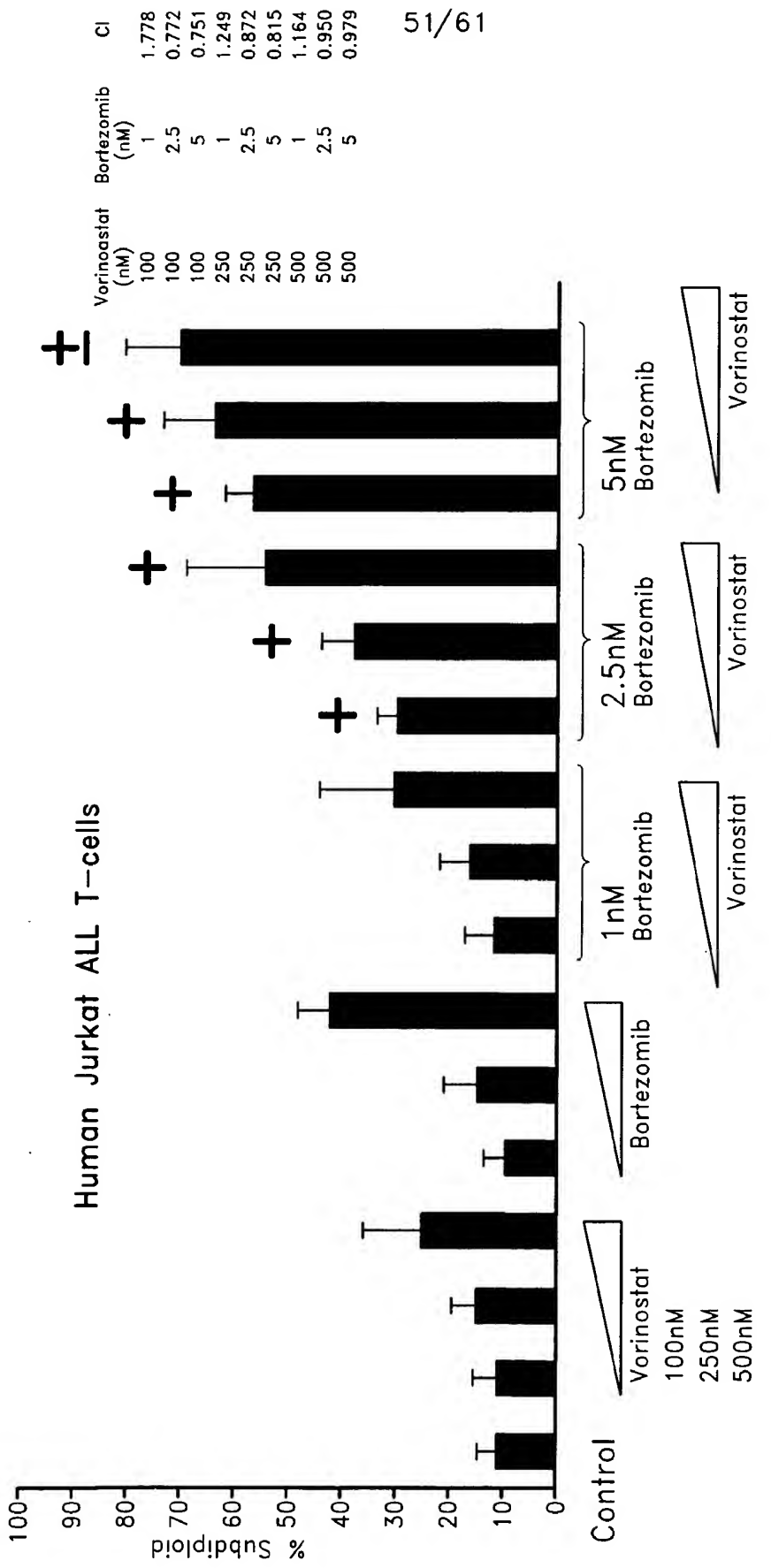

Human Jurkat ALL T-cells
% Subdiploid
100
90
80
70
60
50
40
30
20
10
0
Control
Vorinostat
100nM
250nM
500nM
Bortezomib
1nM
Bortezomib
Vorinostat
2.5nM
Bortezomib
Vorinostat
5nM
Bortezomib
Vorinostat
+
+
+
+
+
+
Vorinoastat (nM)
100
100
100
250
250
250
500
500
500
Bortezomib (nM)
1
2.5
5
1
2.5
5
1
2.5
5
CI
1.778
0.772
0.751
1.249
0.872
0.815
1.164
0.950
0.979

FIG. 31

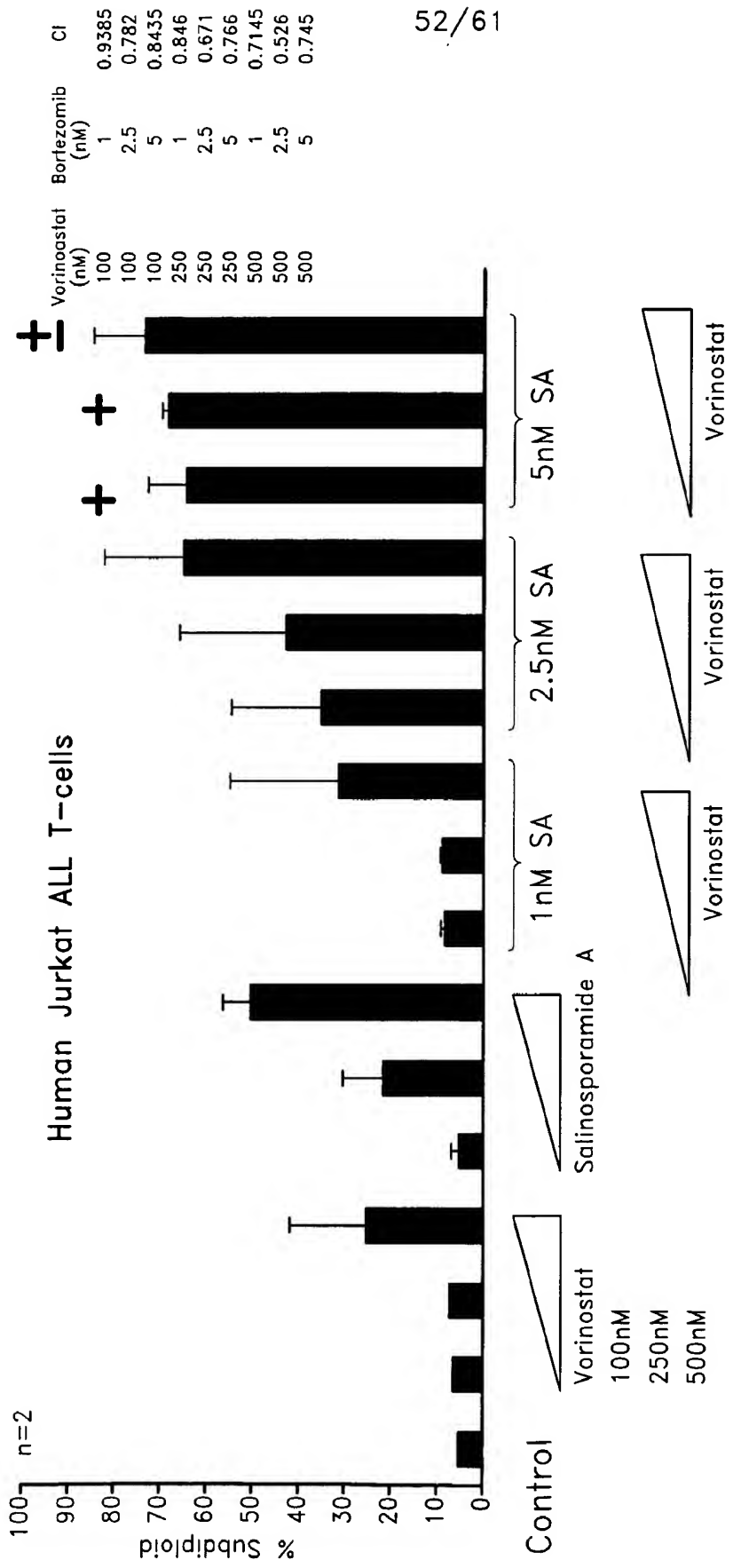

Human Jurkat ALL T-cells
% Subdiploid
n=2
Control
Vorinostat
100nM
250nM
500nM
Salinosporamide A
1nM SA
2.5nM SA
5nM SA
Vorinostat
Vorinostat
Vorinostat
Salinosporamide A (SA)
+
+
+
Vorinostat (nM)
Bortezomib (nM)
CI
100 1 0.9385
100 2.5 0.782
100 5 0.8435
250 1 0.846
250 2.5 0.671
250 5 0.766
500 1 0.7145
500 2.5 0.526
500 5 0.745

FIG. 32

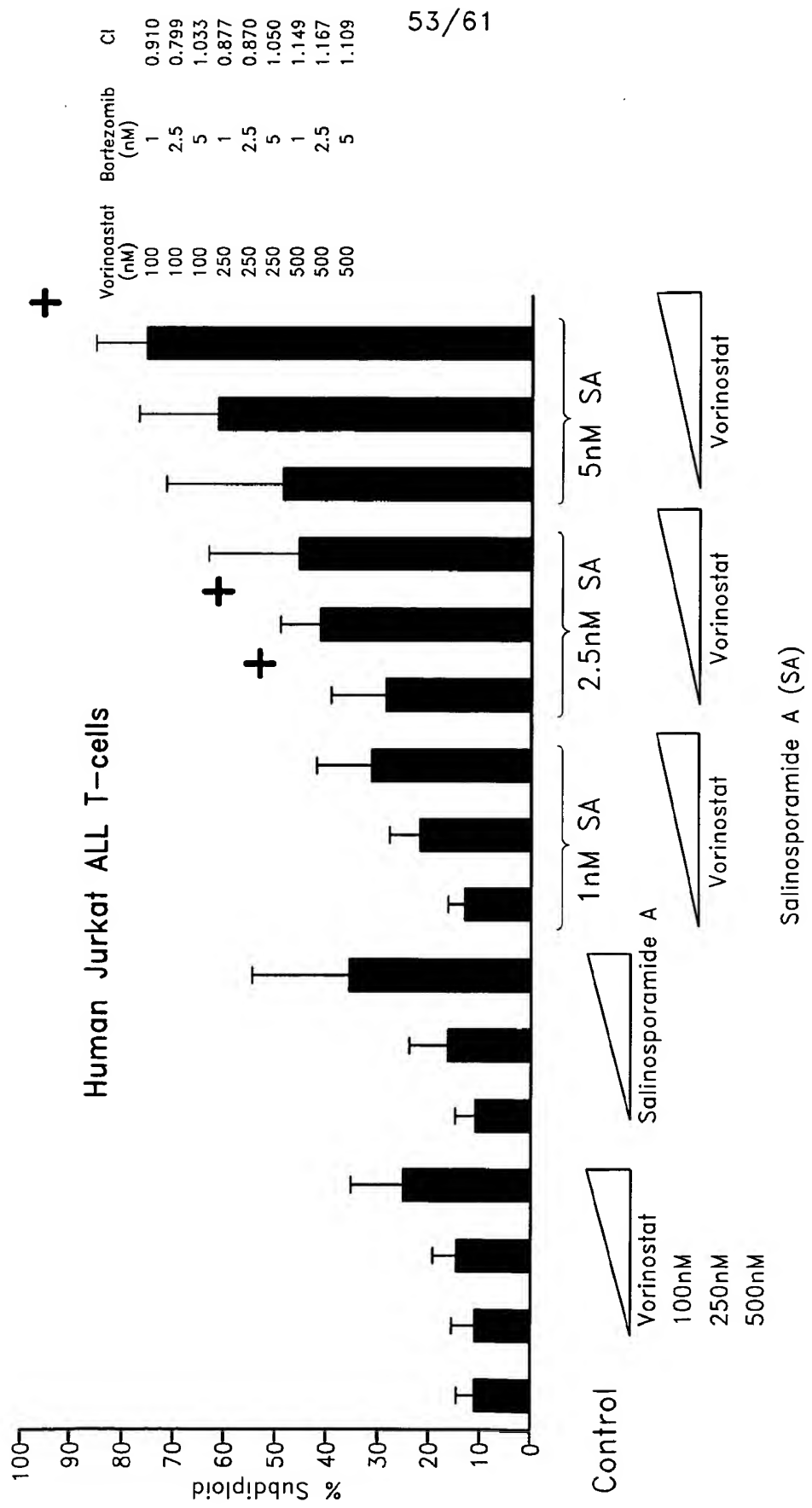

Human Jurkat ALL T-cells
% Subdiploid
100
90
80
70
60
50
40
30
20
10
0
Control
Vorinostat
100nM
250nM
500nM
Salinosporamide A
1nM SA
2.5nM SA
5nM SA
Vorinostat
Vorinostat
Vorinostat
Salinosporamide A (SA)
+
+
+
Vorinoastat (nM)
Bortezomib (nM)
CI
100 1 0.910
100 2.5 0.799
100 5 1.033
250 1 0.877
250 2.5 0.870
250 5 1.050
500 1 1.149
500 2.5 1.167
500 5 1.109

FIG. 33

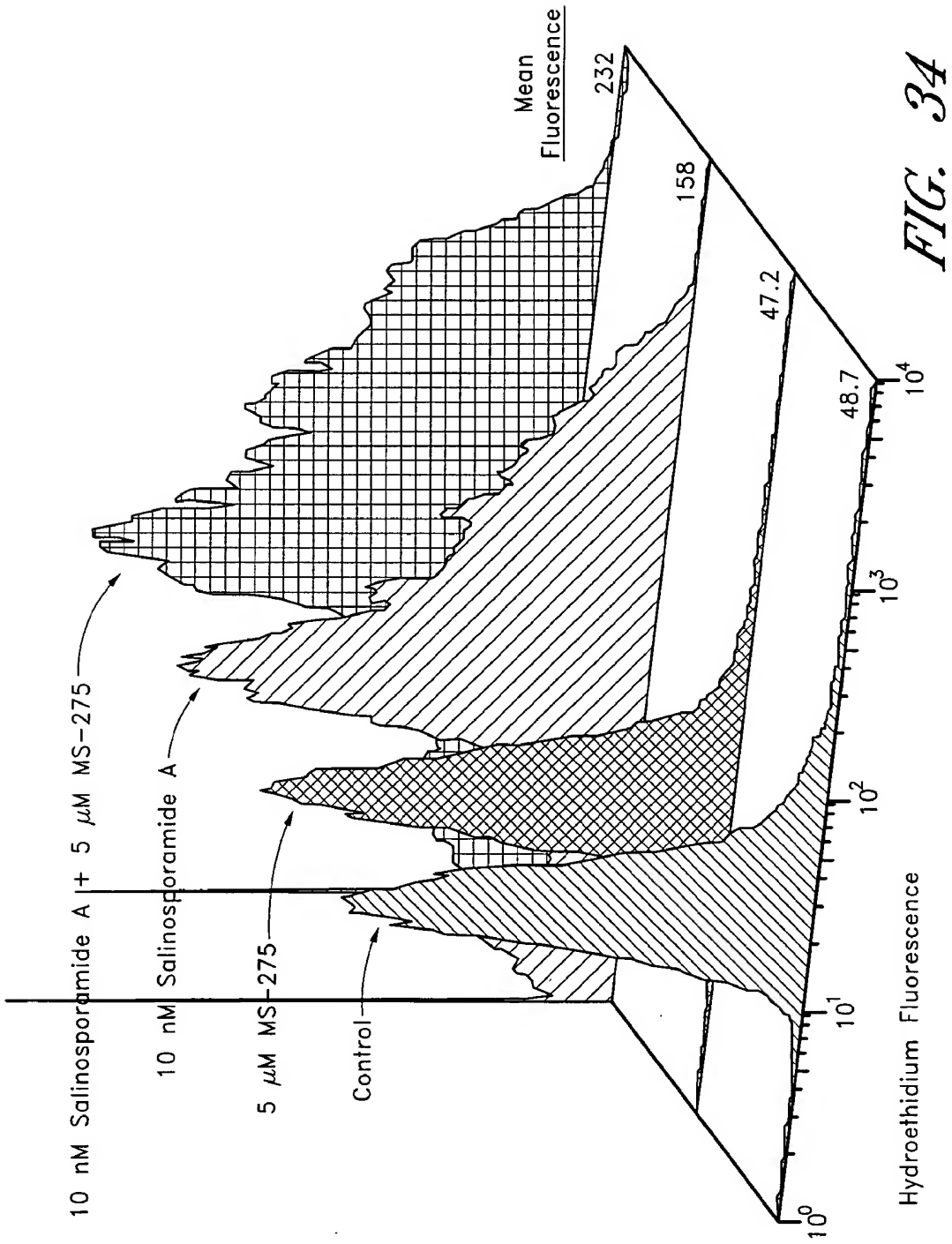
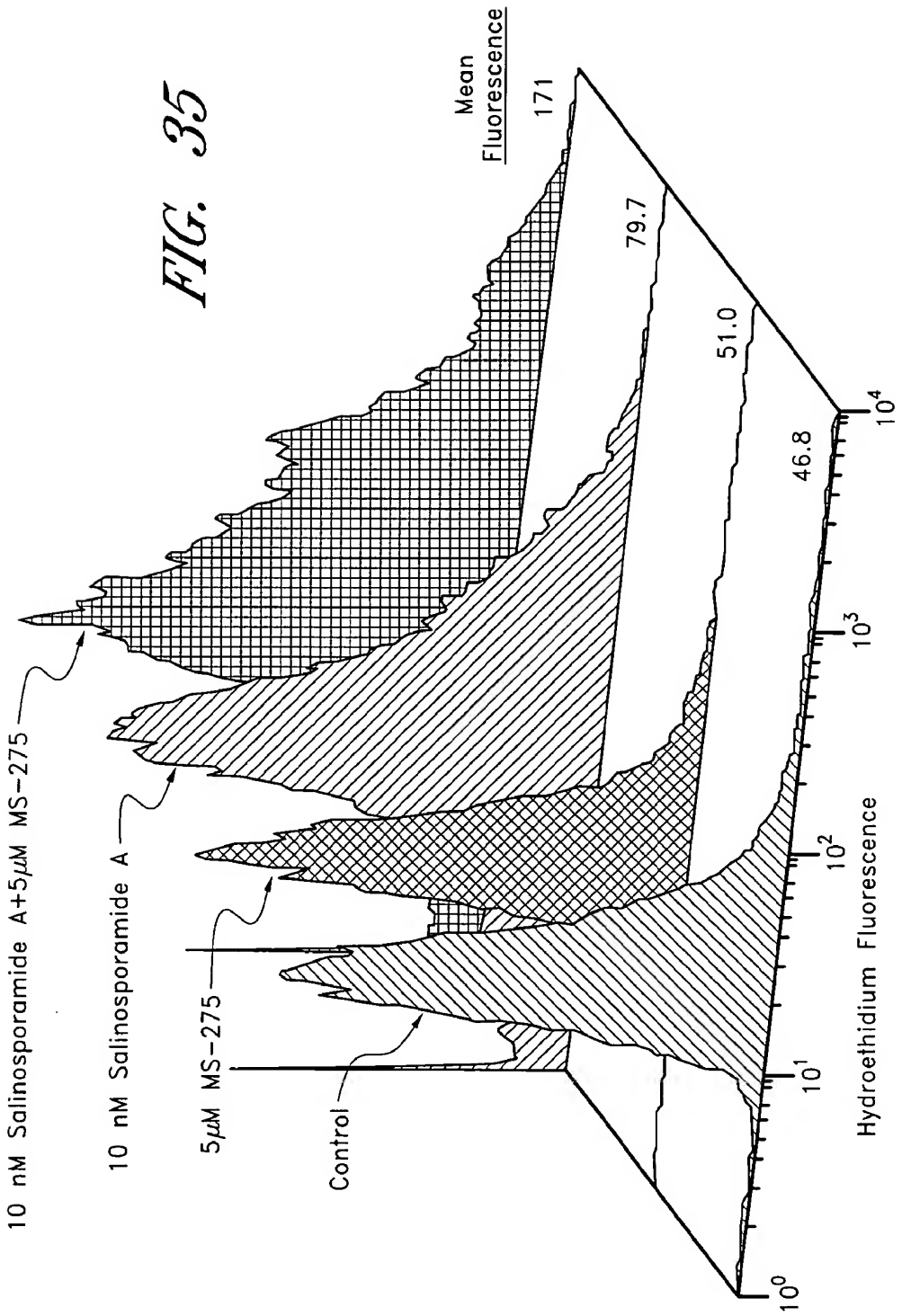

Mean Fluorescence
232
158
47.2
48.7
10^0
10^1
10^2
10^3
10^4
Hydroethidium Fluorescence
Control
5 μM MS-275
10 nM Salinosporamide A
10 nM Salinosporamide A + 5 μM MS-275

FIG. 34

FIG. 35


Mean Fluorescence
171
79.7
51.0
46.8
10 nM Salinosporamide A+5μM MS-275
10 nM Salinosporamide A
5μM MS-275
Control
10^0
10^1
10^2
10^3
10^4
Hydroethidium Fluorescence

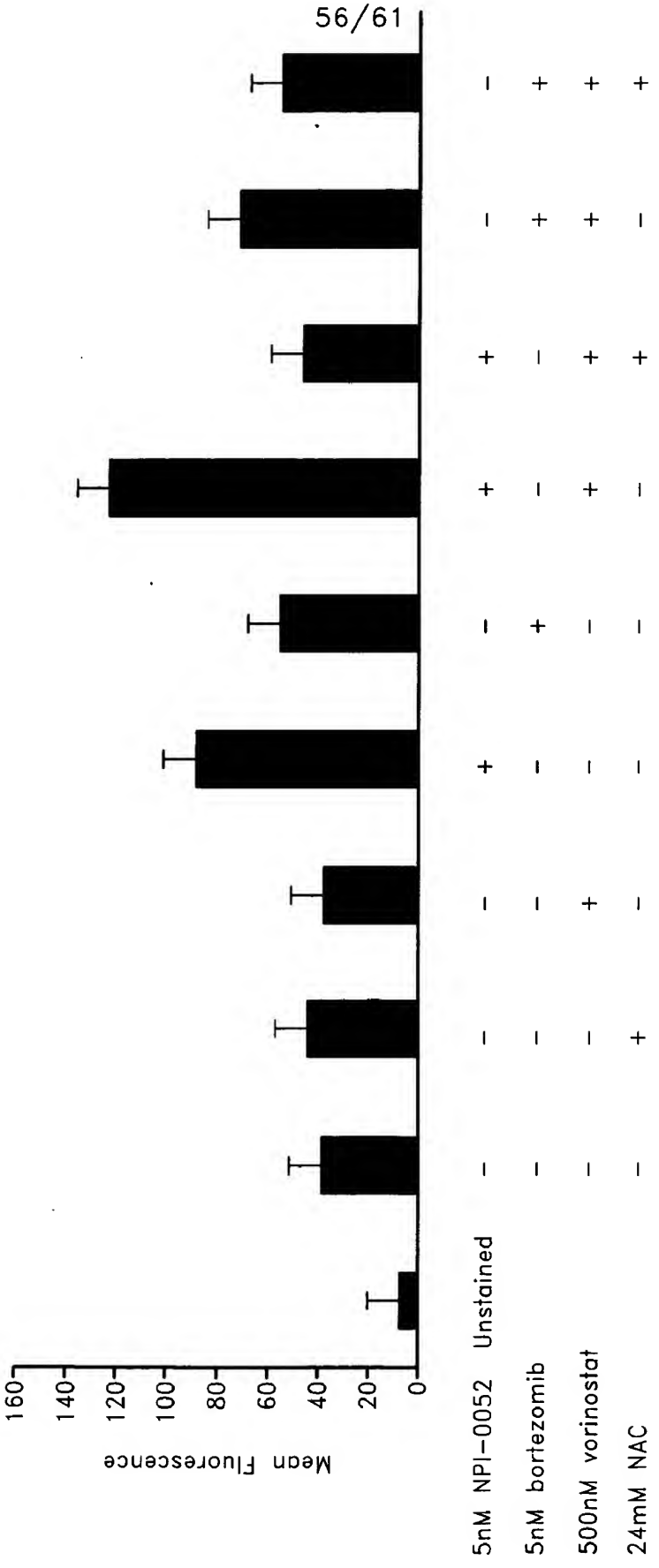

Mean Fluorescence
0
20
40
60
80
100
120
140
160
5nM NPI-0052
5nM bortezomib
500nM vorinostat
24mM NAC
Unstained
NAC: N-acetylcysteine, a free radical scavenger

FIG. 36

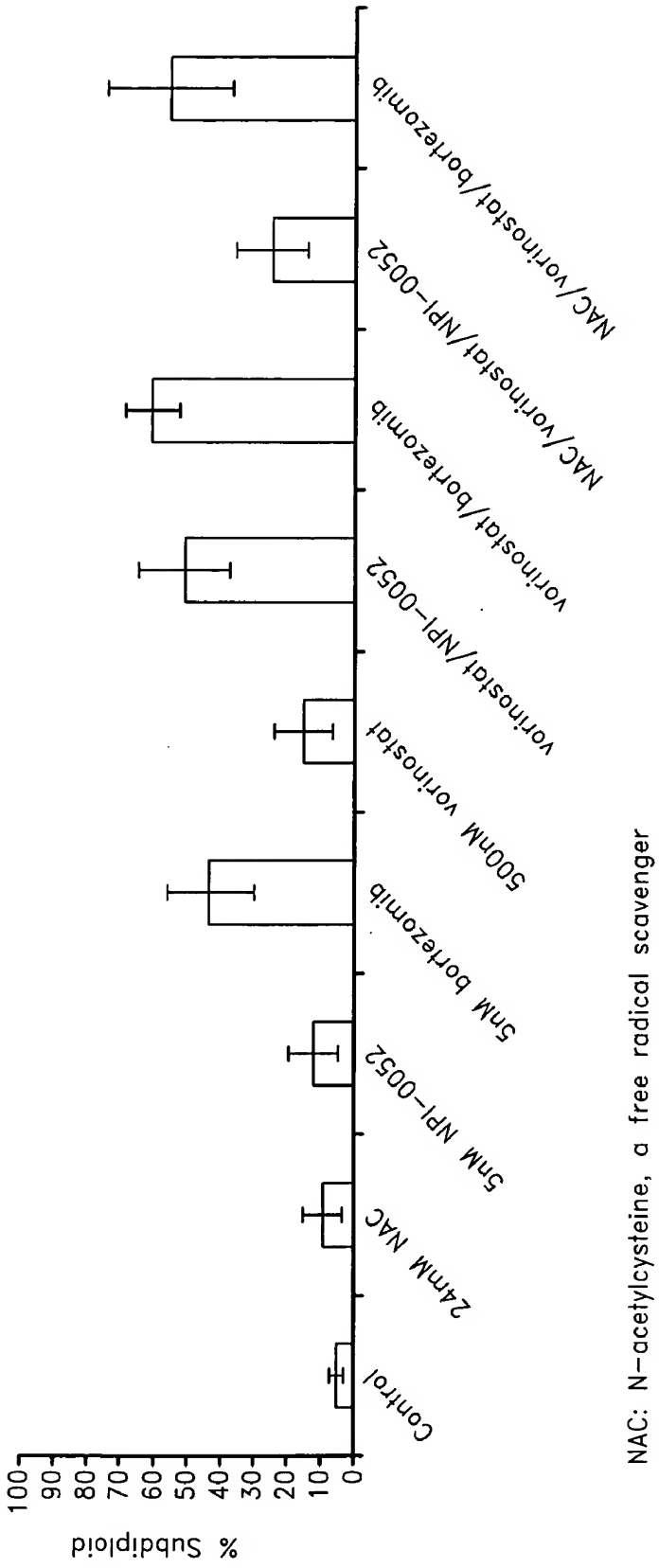

% Subdiploid
100
90
80
70
60
50
40
30
20
10
0
Control
24mM NAC
5nM NPI-0052
5nM bortezomib
500nM vorinostat
vorinostat/NPI-0052
vorinostat/bortezomib
NAC/vorinostat/NPI-0052
NAC/vorinostat/bortezomib
NAC: N-acetylcysteine, a free radical scavenger

FIG. 37

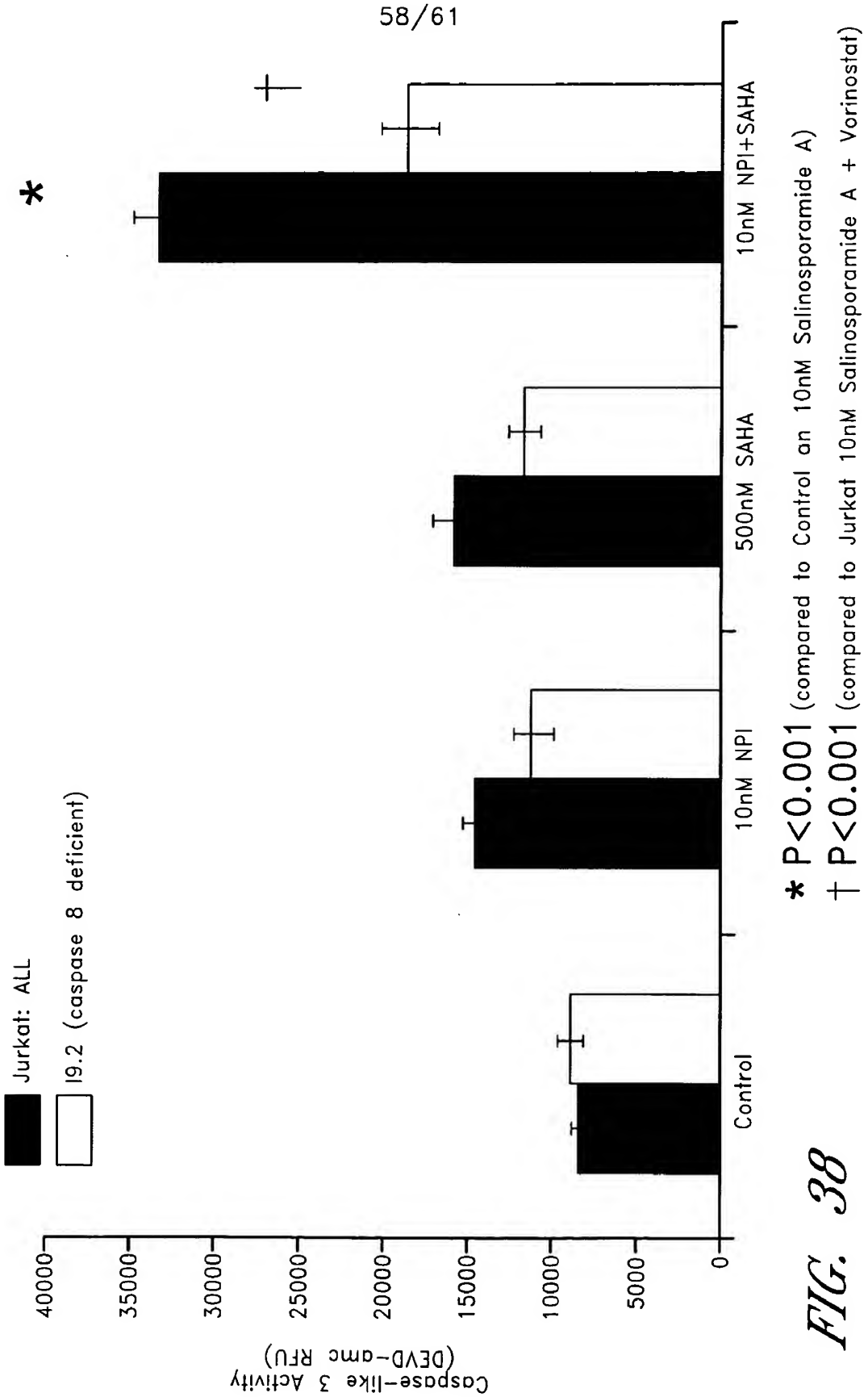

Caspase-like 3 Activity
(DEVD-amc RFU)
40000
35000
30000
25000
20000
15000
10000
5000
0
Jurkat: ALL
I9.2 (caspase 8 deficient)
*
†
Control
10nM NPI
500nM SAHA
10nM NPI+SAHA
* P<0.001 (compared to Control an 10nM Salinosporamide A)
† P<0.001 (compared to Jurkat 10nM Salinosporamide A + Vorinostat)

FIG. 38

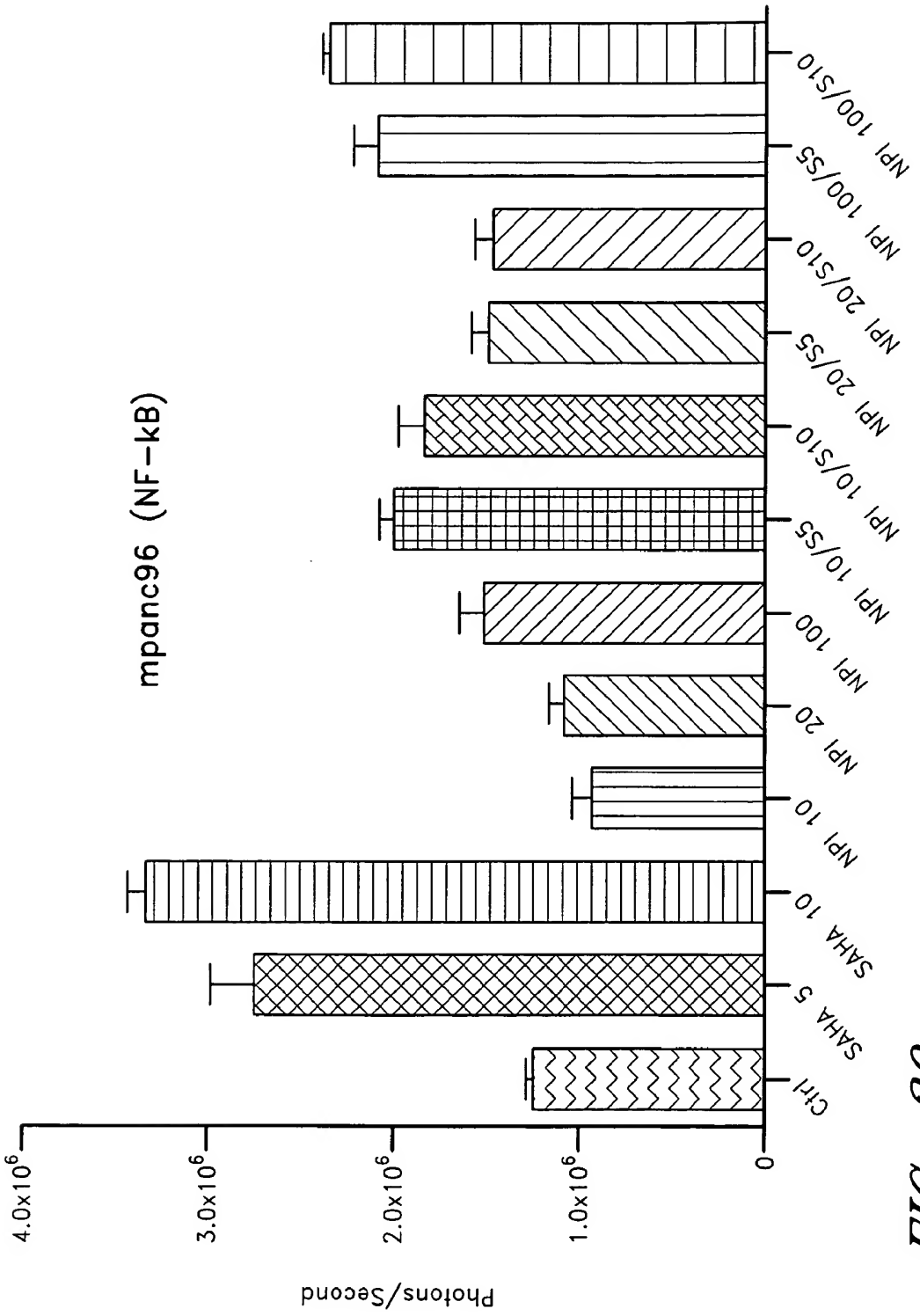
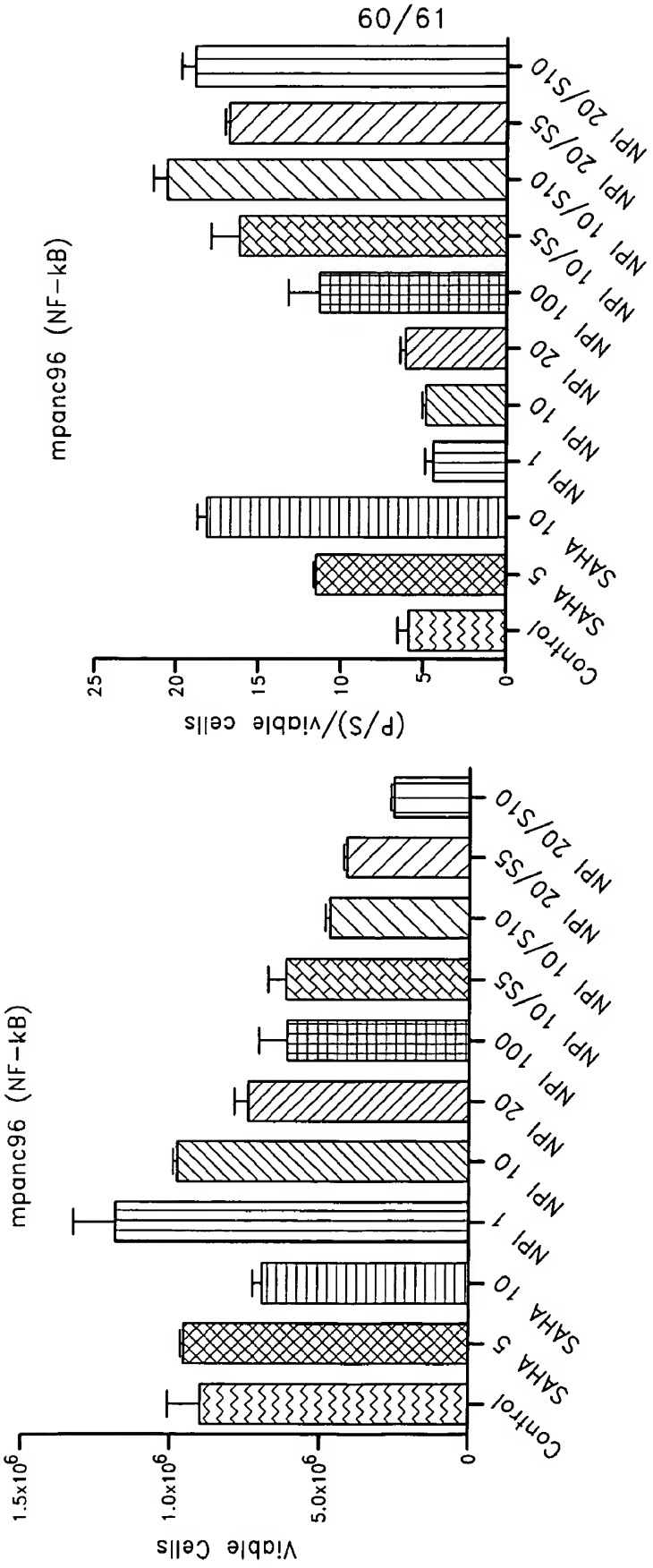

mpanc96 (NF-kB)
Photons/Second
4.0x10^6
3.0x10^6
2.0x10^6
1.0x10^6
0
Ctrl
SAHA 5
SAHA 10
NPI 10
NPI 20
NPI 100
NPI 10/S5
NPI 10/S10
NPI 20/S5
NPI 20/S10
NPI 100/S5
NPI 100/S10

FIG. 39



Total NF-κB activity per well divided by the total viable cell count per well to standardize NF-κB activity per viable cell

FIG. 40

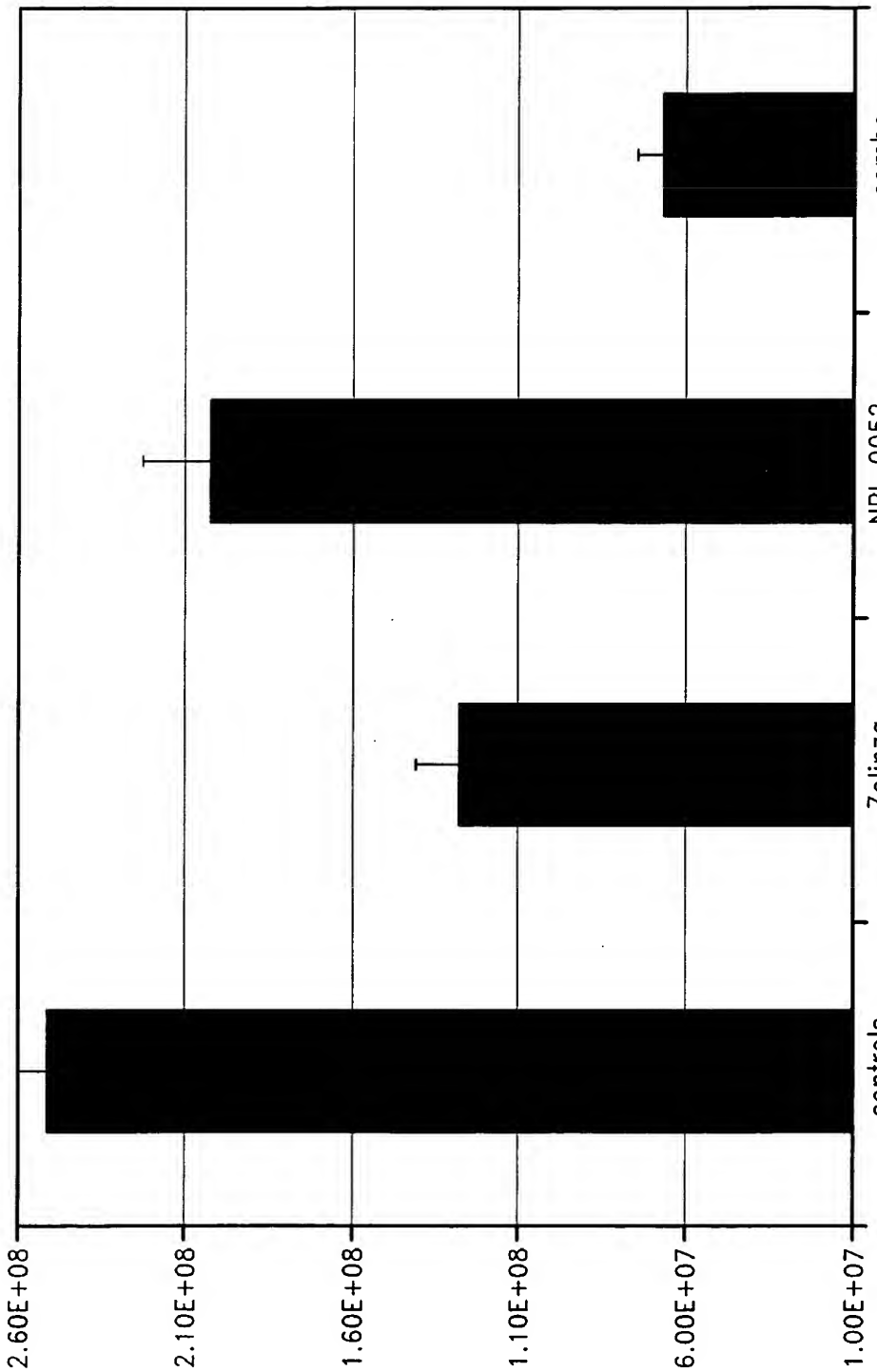

2.60E+08
2.10E+08
1.60E+08
1.10E+08
6.00E+07
1.00E+07
controls
Zolinza
NPI-0052
combo

FIG. 41

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/059592

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/165 A61K31/166 A61K31/167 A61K31/19 A61K31/336
A61K31/40 A61K31/4045 A61K31/407 A61K31/427 A61K31/4406
A61K31/4439 A61K31/473 A61K45/06 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/060819 A (UNIV CALIFORNIA [US]; BONAVIDA BENJAMIN [US]) 8 June 2006 (2006-06-08) page 35, paragraphs 121,122; example 7	1-32
Y	claims 1,6,26	1-32
X	WO 2006/060676 A (DANA FARBER CANCER INST INC [US]; ANDERSON KENNETH C [US]; CHAUHAN DHA) 8 June 2006 (2006-06-08) claims 25-37; example 17	1-32
X	US 2005/288352 A1 (POTTS BARBARA C [US] ET AL POTTS BARBARA CHRISTINE [US] ET AL) 29 December 2005 (2005-12-29) paragraph [0331]; claims 1-39.	1-32

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2008	15/09/2008

Name and mailing address of the ISA/	Authorized officer
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International application No
PCT/US2008/059592

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